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(57) Abstract

The present disclosure provides specific nucleotide sequences and diagnostic methods for prototype serogroup A, B, C, Y and W–135 strains of *Neisseria meningitidis*. Due to capsule switching *in vivo*, closely related virulent meningococcal clones may not be recognized by traditional serogroup–based surveillance, and these strains can escape vaccine–induced or natural protective immunity by capsule switching. The invention provides recombinant meningococcal strains, recombinant DNA constructs and immunological preparati ns useful as diagnostic probes for detection and diagnosis of meningococcal diseases, screening for specific meningococcal serogroups and broad based immunizations with multivalent capsular polysaccharide conjugate vaccines.

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SEROGROUP-SPECIFIC NUCLEOTIDE SEQUENCES IN THE MOLECULAR TYPING OF BACTERIAL ISOLATES AND THE PREPARATION OF VACCINES THERETO

This invention was made, at least in part, with funding from the United States National Institute of Allergy and Infectious Diseases, Grant No. AI40247-01. Accordingly, the United States government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

This invention relates generally to the nucleotide sequences of serogroup-specific capsular polysaccharides genes and their use in a method for typing of serogroups of pathogenic bacteria, in particular *Neisseria meningitidis,* and further, relates to capsule gene switching in recombinant strains and the detection thereof.

Contagious outbreaks of epidemic diseases constitute public health emergencies requiring rapid treatment and chemoprophylaxis of contacts. Vaccination of the population at risk can be considered if disease cases continue to occur. However, asymptomatic carriage of pathogens in humans is common and some of the adult population may be immunized from previous outbreaks. The factors leading from acquisition of the organism to invasive disease point to a clonal origin of the outbreaks and to an enhanced virulence or altered antigenicity of a particular clone.

Neisseria meningitidis is a leading worldwide cause of meningitis and rapidly fatal sepsis in otherwise health individuals [Apicella, M.A. (1995) in *Principles and Practice of Infectious Diseases*, eds. Mandell, G.L., Douglas, R.G., and Bennett, J.E., Churchill Livingstone, New York, pp. 1896-1909]. In excess of 350,000 cases of meningococcal disease were estimated to have occurred in 1995 [WHO Report (1996) WHO, Geneva, ISBN 92 4 1561823]. The problem of meningococcal disease is emphasized by the recurrence of major epidemics due to serogroups A, B, and C *N. meningitidis* over the last 20 years, such as: the devastating serogroup A outbreak in sub-Saharan Africa in 1996 [WHO (1996) *Meningitis in Africa. The constant challenge of epidemics.* WHO 21:15 March]; the recent dramatic increases in the incidence of serogroup B and C meningococcal disease in parts of

North America [CDC (1995) *MMWR* **44**:121-134; Jackson, L.A. et al. (1995) *JAMA* **273**:390-394; Wahlen, C.M. et al. (1995) *JAMA* **273**:383-389]; and the emergence in Europe and elsewhere of meningococci with decreased susceptibility to antibiotics [Campos, J. et al. (1992) *J. Infect. Dis.* **166**:173-177].

Differences in capsular polysaccharide structure determine the meningococcal serogroups [Liu, T.Y. et al. (1971) *J. Biol. Chem.* **246**:2849-58; Liu, T.Y. et al. (1971) *J. Biol. Chem.* **246**:4703-12]. Meningococci of serogroups B, C, Y, and W-135 express capsules composed entirely of polysialic acid or sialic acid linked to glucose or galactose [Liu, T.Y. et al. (1971) *J. Biol. Chem.* **246**:4703-12; Bhattacharjee, A.K. et al. (1976) *Can. J. Biochem.* **54**:1-8], while the capsule of group A *N. meningitidis* is composed of *N*-acetyl mannosamine-1-phosphate [Liu, T.Y. et al. (1971) *J. Biol. Chem.* **246**:2849-58]. The currently available capsular polysaccharide vaccines for serogroups A, C, Y, or W-135 *N. meningitidis* are effective for control of meningococcal outbreaks in older children and adults. However, because of poor immunogenicity in young children and short-lived immunity [Zollinger, W.D. and Moran, E. (1991) *Trans. R. Soc. Trop. Med. Hyg.* **85**:37-43], these vaccines are not routinely used for long-term prevention of meningococcal disease. In the case of group B *N. meningitidis*, whose (α2→8)-linked polysialic capsule is an immunotolerized self antigen, a reliable polysaccharide vaccine is not yet available. However, rapid progress is being made in development of polysaccharide-protein conjugate vaccines.

In some epidemic settings, simultaneous or closely-linked meningococcal outbreaks have occurred in the same population due to different serogroups [Sacchi, C.T. et al. (1994) *J. Clin. Microbiol.* **32**:1783-1787; CDC (1995) *MMWR* **44**:121-134; Krizova, P. and Musilek, M. (1994) *Centr. Eur. J. Publ. Hlth* **3**:189-194]. Further, Caugant et al. [Caugant, D.A. et al. (1986) *Proc. Natl. Acad. Sci. USA* **83**:4927-4931; Caugant, D. A. et al. (1987) *J. Bacteriol.* **169**:2781-2792] and others have noted that meningococcal isolates of different serogroups may be members of the same enzyme type (ET)-5, ET-37 or ET-4 clonal complexes.

Since 1993, the number of cases of serogroup B meningococcal disease in Oregon and adjacent counties in Washington State has doubled. The U.S. serogroup B meningococcal strains are closely related to the ET-5 complex. ET-5 complex strains have been responsible for major epidemics in Norway, Iceland, Cuba and South America over the last twenty years [Caugant, D.A. et al. (1986) *Proc. Natl. Acad. Sci. USA* **83**:4927-4931; Sierra, G.V. et al.

(1991) *NIPH Annals* 14:195-207; Sacchi, C.T. et al. (1992) *J. Clin. Microbiol.* 30:1734-1730]. Since 1994, cases of serogroup C meningococcal disease due to ET-5 complex strains were also noted in Oregon and Washington State. There exists an urgent need to understand the genetic basis for meningococcal capsule expression and to rapidly diagnose strains of serogroup A and other serogroups of *N. meningitidis*.

SUMMARY OF THE INVENTION

The present invention provides the nucleotide sequence of the intergenic region separating *ctrA* from the biosynthesis operon (*synA*-D,E,F,G) of a serogroup A *N. meningitidis*. Whereas in serogroups B, C, Y and W-135 *N. meningitidis*, the intergenic region separating *ctrA* from the biosynthesis operon (*synA*-D,E,F,G) is 134 bp and contains the *ctrA*-D promoter as well as the divergent biosynthesis operon promoter and other transcriptional regulatory elements, in serogroup A *N. meningitidis* the intergenic region is 218 bp in length and does not share any homology with the 134 bp region found in the sialic acid capsular serogroups.

This invention also provides evidence that the DNA located between *ctrA* and *galE* in serogroup A *N. meningitidis* is a cassette containing four genes ORF1-ORF4 responsible for the production of serogroup A capsule from UDP-N-acetylglucosamine. The predicted amino acid sequences encoded by ORF1-ORF4 are also provided by the present invention.

Further, according to this invention, the ORF1-ORF4 genes are divergently co-transcribed from overlapping promoters located in a short intergenic region separating the capsule biosynthetic and transport operons. Mutagenesis of these genes demonstrates the involvement of these genes in serogroup A capsule production.

The invention also provides a model in which meningococcal capsular serogroups are determined by specific genetic biosynthesis cassettes that insert between the *ctrA* operon and *galE*. In specific embodiments, it is demonstrated for serogroup A meningococci that the cassettes determining specificity of serogroups can recombine to switch the type of capsule and serogroup expressed. Such information is critical to the design of improved group A and other meningococcal vaccines and to the understanding of the molecular basis of serogroup A pathogenesis.

It is an object of the present invention to provide strains of *N. meningitidis* of a particular serogroup transformed *in vitro* to express a capsule polysaccharide marker of a different meningococcal strain serogroup. In a particular embodiment are provided prototype serogroup C, Y and W-135 meningococcal strains transformed *in vitro* with DNA comprising the *synD* of the serogroup B strain NMB. According to the present invention, conversion from one sialic acid expressing capsule serogroup to another can be accomplished by homologous recombination of the sequences encoding the serogroup-specific capsule polymerase. Such recombinant *N. meningitidis* strains are provided according to the invention as genetically engineered *in vitro* recombinations.

Also provided by the present invention are *Neisseria meningitidis* mutant serogroup strains which express different non-isogeneic capsular polysaccharides due to homologous recombination of the sequences encoding the serogroup-specific capsule polymerase. Specifically exemplified herein is a mutant *N. meningitidis* strain 1070 (serogroup B, ET-301) in which genetic markers are isogeneic to serogroup B except for the capsule polysaccharide, which is a serogroup C marker. Such meningococcal isolates comprise a recombinant or switched capsule gene and, in a particular embodiment, a switching or recombination event occurred from a serogroup B to a serogroup C capsule biosynthetic gene. Such recombinant *N. meningitidis* strains are provided according to the invention as naturally-occurring *in vivo* recombinant isolates.

It is also an object of the invention to provide meningococcal serogroup-specific capsule genes encoding characteristic capsular polysaccharide virulence determinants. In specific embodiments of the invention are provided capsule biosynthetic gene preparations of prototype serogroups A, B, C, Y and W-135, each serogroup-specific gene encoding a biosynthetic enzyme for specific and distinguishing capsular polysaccharide.

It is an additional object of the invention to provide cloned DNA molecules which can be used to introduce an additional non-isogeneic capsular polysaccharide virulence determinant into strains of *N. meningitidis*. In a particular embodiment, the cloned DNA fragment containing the stable Tn916 insertion in the *synD* of the serogroup B *N. meningitidis* strain NMB was used to introduce the gene for the serogroup B ($\alpha 2 \rightarrow 8$)-linked capsule polysialyltransferase into other meningococcal strains to produce novel immunotypes. More generally, a cloned DNA fragment containing a stable insertion of a polysialyltransferase gene

of a specific serogroup strain can be used to introduce the corresponding capsular polysaccharide determinant into serologically different strains to produce novel immunotypes. This invention also contemplates that multiple non-isogeneic capsular polysaccharide virulence determinants can be introduced into serologically different meningococcal strains.

It is an added object of the present invention to provide protective immunity from virulent meningococcal strains that can escape vaccine-induced or natural protective immunity by capsule switching. In particular embodiments, the invention provides multivalent vaccines which effectively anticipate capsule switching events. According to the invention, broad immunization with capsular polysaccharide vaccines effective against all major capsular serogroups can be used to control epidemics and endemic disease or vaccines for particular serogroups can be used in geographic areas (or for travelers to areas) where the cognate serogroup is endemic.

It is yet another object of the invention to provide a method for diagnostic detection and serogroup typing of *N. meningitidis* strains, especially those of Group A. This method is a nucleic acid amplification (e.g., PCR) method or nucleic acid hybridization method based on (a) the specific nucleotide and encoded amino acid sequences of serogroup-specific capsular polysaccharide determinants and (b) oligonucleotide primers designed to anneal to specific capsule polymerase sequences. This method of the invention was particularly exemplified in the typing of *N. meningitidis* serogroups A,B,C, Y and W-135. This nucleic acid amplification method of the invention, based on the use of discriminatory primers derived from serogroup-specific nucleotide sequences (Sequo-grouping), offers advantages over current methods of diagnostic detection of serogroup typing in (a) being independent of the need to grow pathogenic organisms for immunological analyses, (b) being capable of being performed directly on clinical specimens, e.g., blood cerebrospinal fluid, with the need to isolate pathogenic organisms, (c) being capable of detecting nucleotide sequences in not only living but also nonliving or nonviable organisms, (d) reducing the exposure of personnel to large volumes of pathogenic bacteria, (e) reducing the cost per serogroup analysis, and (f) improving significantly the accuracy of the serotyping method. This method is particularly preferred as an easy, convenient and rapid screening method for the presence of virulent strains of encapsulated pathogens.

Also provided are compositions and immunogenic preparations including but not limited to vaccines, as specifically exemplified, comprising at least one capsular polysaccharide derived from one serogroup strain of *N. meningitidis* and at least one capsular polysaccharide from a different meningococcal serogroup strain, and a suitable carrier therefor are provided. Alternatively, the immunogenic composition can comprise cells of at least two different serotype strains of the specifically exemplified *N. meningitidis* strains and a suitable carrier.

BRIEF DESCRIPTION OF THE FIGURES

Figs. 1A-1D present schematically molecular analysis of capsule biosynthesis and membrane transport genes in prototype isolates of serogroup A, B, C, Y and W-135 *N. meningitidis*. Fig. 1A illustrates the genetic basis for serogroup B meningococcal capsular polysaccharide. Meningococcal capsules are produced by genes encoded by the 24 kb *cps* gene complex comprising five regions: E, C, A, D, and B. In serogroup B, four capsular biosynthetic genes (*synX-D)* are found in region A and are transcribed as an operon. Region C, adjacent to region A, contains 4 polycistronic genes, *ctrA-D*, encoding proteins which transport the phospholipid-substituted polysialic acid across the inner and outer membranes. The *ctr* genes are transcribed in the opposite orientation from the *syn* biosynthetic genes of region A, but utilize the same 134 bp promoter region [Swartley et al. (1996) *J. Bacteriol.* **178**:4052-4059].

Fig. 1B illustrates the biosynthetic pathway for the production of serogroup B capsule; *SynX* is either the N-acetyl-D-glucosamine-6-phosphate 2-epimerase which produces N-acetyl-D-mannosamine-6-phosphate or a specific phosphatase which converts N-acetyl-D--mannosamine-6-phosphate into N-acetyl-D-mannosamine; *SynB* is the CMP-N-acetylneuraminic acid (NANA) synthetase; *SynC* is the NANA synthetase; and *SynD* is the polysialyltransferase responsible for (α2→8)-linked polysialic acid chain polymerization and elongation.

Fig. 1C illustrates Southern DNA hybridization showing *ctrA* homology in serogroups A (strains F8229, F8239), B (strains NMB, 1070 [B-301*]), C (strains FAM 18, 1205 [C-301*], 1843 [C-301]), Y (strain GA0929), and W-135 (strain 6083) of *N. meningitidis*.

Chromosomal DNA from each of the strains was prepared, digested with *Cla*I, electrophoresed through a 1.2% agarose gel and transferred to a nylon membrane. The membrane was then probed with a 150 bp digoxigenin-labeled PCR product derived from the 5'-end of the serogroup B *ctrA* gene. *N. lactamica* and *N. gonorrhoeae* (GC) showed no hybridization. Molecular weight size standards (Boehringer Mannheim Biochemical) flank the chromosomal digests.

Fig. 1D illustrates PCR amplification of *ctrA* and *synX-synD* from serogroups A (strain F8239), B (strain NMB), C (strain FAM18), W-135 (strain 6083), and Y (strain GA0929) *N. meningitidis* using oligonucleotide primers derived from the individual gene sequences of serogroup B prototype strain NMB. Kb DNA ladders (BRL) flank the gel.

Fig. 2 presents multiple nucleotide sequence alignment of the 3' end of *synC* and downstream sequence in serogroups B (strain NMB) [SEQ ID NO:1], C (strain FAM18) [SEQ ID NO:2], W-135 (strain GA1002) [SEQ ID NO:3], and Y (strain GA0929) [SEQ ID NO:4] *N. meningitidis,* pretty multiple sequence comparison program (GCG). In the consensus sequence [SEQ ID NO:5], consensus nucleotide matches (3 or more identical) at each position are indicated in upper case type, while differences from consensus are indicated by lower case type. Dots (...) indicate gaps introduced by the analysis program to facilitate alignment. The *synC* termination codon (TAA) and the *synDIElF* start codons (ATG) are shown in bold type. The location of an IS1301 element located downstream of the *synC* gene in the otherwise identical sequence of a second serogroup W- 135 strain, 6083, is shown in the GA 1002 sequence by an A^. The complete sequence of *synE* derived from serogroup C strain FAM18 is available through the GenBank/EMBL nucleic acid database under accession number U75650.

Fig. 3A-3B summarize genetic analyses of serogroup B301 (strains 1070 and 1069) and C301 (strains 1205, 1198 and 1204) *N. meningitidis* recovered from the Oregon/Washington State outbreak. Fig. 3A illustrates the nucleotide sequence alignment of the 3'-end of *synC* and downstream sequence in serogroup B strains NMB (SEQ ID NO:1, positions 1-277) and 1070 (B-301#1) [SEQ ID NO:6], and serogroup C strains FAM18 (SEQ ID NO:2, positions 1-275) and 1205 (C-301#1) [SEQ ID NO:7] (Pretty multiple sequence comparison program of the Genetics Computer Group [GCG] sequence analysis package version 7.3.1 UNIX. The *synC* termination codon (TAA) and the *synDIE* start codons (ATG)

are indicated in bold type. The consensus sequence corresponds to SEQ ID NO:29. Fig. 3B-1 to 3B-3 illustrate nucleotide polymorphisms of the B301, C301 and other meningococcal strains. Fig. 3B-1 illustrates polymorphisms within a 909 bp PCR product containing the 5'-ends of both *ctrA and synX* and the 134 bp intergenic region separating these two genes (bps 1-319 are the 5' end of *ctrA*, bps 320-453 are the 134 bp intergenic region, and bps 454-909 are the 5' end of *synX*). Fig. 3B-2 illustrates polymorphisms within a 238 bp PCR product amplified from the 330 bp *fkbp* gene, and an 803 bp PCR product amplified from the 1128 bp *rec*A gene. Regions were sequenced from strains 1070 (B301 # 1) (B), 1069 (B301 #2) (B), FAM18 (C), 1205 (C301 # 1) (C), 1198 (C301#2) (C), 1204 (C301#3) (C), NMB (B), GA1002 (W-135), F8239 (A), GA0929 (Y), and GA1002 (W-135) and compared to the sequence of other neisserial strains. The sequence of strain 1070 (B301#1) was used as the master sequence. Differences from the master sequence are indicated at the nucleotide positions within *FKBP*, *recA*, or the *ctrA-synX* PCR product, identity at a given position is indicated by a dash (-) and deleted nucleotides are shown by dots (...).

Fig. 4 presents a schematic illustrating the arrangement of four ORFs located between *ctrA* and *galE*. The four ORFs are transcribed in the opposite direction with respect to *ctrA*.

Fig. 5 presents the nucleotide sequence [SEQ ID NO:35] of the 218 bp intergenic region separating the start codons for the serogroup A *ctrA* and ORF1 loci. The start points and direction of transcription of the ORF1 and *ctrA* mRNA are indicated by t_i and a right- or left-hand arrow, respectively. Predicted -10 and -35 promoter binding sequences are indicated, as well as the putative Shine-Dalgarno ribosome binding sites (RBS). The predicted initiation codons for *ctrA* and ORF1 are shown in boxes.

Fig. 6 presents RT-PCR of mRNA prepared from wild-type serogroup A strain F8229 for detection of ORF1-ORF4 polycistronic transcripts. Lane 1 contains the 1 kilobase ladder (Gibco-BRL). Lane 2 is the positive control PCR amplification of ORF1-ORF4 using F8229 chromosomal DNA as the template and primers SE46 and SE61 (Table 2). Lane 3 contains the RT-PCR result using primers SE46 and SE61. Lane 4 contains the RT-PCR negative control reaction for which conditions were identical to those used in lane 3, with the exception that RT was not added to the reaction mixture. DNA size standards in base pairs (bp) are indicated.

Figs. 7A and 7B present autoradiograph results showing primer extension products for the meningococcal serogroup A genes *ctrA* and ORF1. Primer extension reactions were loaded alongside standard double-stranded DNA sequencing reactions (load orientation of G, A, T, C) obtained by sequencing *ctrA* and ORF1 control DNA templates using the extension primers SE40 (*ctrA*) and SE41 (ORF1). The DNA sequence surrounding the primer extension bands have been expanded. The nucleotides corresponding to the putative start points of transcription have been circled.

DETAILED DESCRIPTION OF THE INVENTION

The following definitions are given in order to provide clarity as to the intent or scope of their usage in the specification and claims.

The term *genetically stable*, as used herein, relates to a mutant that does not revert to the wild-type phenotype at a significant frequency, with reversion occurring at a frequency of less than 10^{-6}, preferably less than 10^{-8}, and more preferably less than 10^{-10}.

The terms *serogroup marker* or *particular serogroup marker* or *marker of a serogroup* or *serologically-distinguishing marker*, as used herein, relate to a capsular polysaccharide synthesized specifically by a particular serogroup strain of *Neisseria meningitidis*. For example, the capsular polysaccharide genes, *synD, synE, synF* and *synF*, differ from each other at a nucleotide level and are only found in the chromosomes of their particular serogroup. Thus, the presence of a specific capsular polysaccharide gene in a neisserial strain is used as a marker or a diagnostic to identify or label or type the serogroup of the meningococcal strain.

The terms *capsular switching* or *capsular recombination*, as used herein, relate to the exchange or substitution or recombination of a capsular polysaccharide gene specifying a particular serogroup with a corresponding capsular polysaccharide gene specifying a different serogroup.

The terms *stringent hybridization conditions* or *hybridization under stringent conditions* or *selective hybridization*, as used herein, relate to experimental conditions

understood in the art for a given probe length and nucleotide composition and capable of hybridizing under stringent conditions means annealing to a subject nucleotide sequence, or its complementary strand, under standard conditions (i.e., high temperature and/or low salt content) which tend to disfavor annealing of unrelated sequences. As specifically exemplified, "conditions of high stringency" means hybridization and wash conditions of 65°-68°C, 0.1 X SSC and 0.1% SDS (indicating about 95-100% nucleotide sequence identity/similarity). Hybridization assays and conditions are further described in Sambrook et al. (1989).

To assess the molecular epidemiology, serogroup B and C meningococcal strains from the Pacific Northwest outbreak were examined by ET typing, serotyping, and PFGE. The strains examined included thirty-five ET-5 complex strains consecutively isolated during 1994 in Oregon, of which 29 were serogroup B and 6 were serogroup C, and five serogroup B ET-5 complex strains recovered in 1994-1995 from Washington State and California. The 1994 Oregon isolates were typed. Approximately 88% of serogroup B isolates were found to be ET-5 complex strains and approximately 17% of the C isolates were ET-5 complex strains. None of the strains were from case-clusters or from epidemiologically-linked patients. All were ET-301 (a member of the ET-5 complex). All except one were serotype 4 or 15, all were immunotype 1.7, 16 and all except one expressed the L3,7,9 LOS immunotype. One predominant PFGE pattern (A) was seen in these isolates. None of the isolates differed from the predominant PFGE A pattern by more than three bands, indicating the isolates were closely related (Tenover et al. (1995) *J. Clin. Microbiol.* **33**:2233-2239).

These data correlated well with similar data on other strains of this outbreak isolated in 1993-1996 and showed identity or close-relatedness to the ET-5 serogroup B strains causing the epidemic disease in the Pacific Northwest. In addition, the serogroup C strains isolated were identical to the dominant serogroup B strains by these molecular epidemiologic markers. These data indicated that the epidemic meningococcal clone causing the outbreak in the Pacific Northwest expressed either serogroup B [(α2→8)-linked polysialic acid] or serogroup C [(α2→9)-linked polysialic acid] capsular polysaccharide. Moreover, the outbreak strains were distinct by ET typing, serotyping, subtyping, and PFGE from serogroup B and C meningococcal disease isolates recovered from other parts of the country during this time.

The genetic basis for serogroup B meningococcal capsule biosynthesis and membrane translocation [Frosch, M. et al. (1989) *Proc. Natl. Acad. Sci. USA* **86**:1669-1673; Edwards, U. et al. (1994) *Mol. Microbiol.* **14**:141-149; Swartley, J.S. and Stephens, D.S. (1994) *J. Bacteriol.* **176**:1530-1534; Ganguli, S. et al. (1994) *J. Bacteriol.* **176**:4583-4589; Edwards, U. and Frosch, M. (1992) *FEMS Microbiol. Lett.* **96**:161-166; Frosch, M. et al. (1991) *Mol. Microbiol.* **5**:1251-1260; Frosch, M. et al. (1992) *Infect. Immun.* **60**:798-803; Swartley, J.S. et al. (1996) *J. Bacteriol.* **178**:4052-4059; and Hammerschmidt, S. et al. (1996) *EMBO J.* **15**:192-198] is summarized in Fig. 1A. The *cps* gene complex of group B *N. meningitidis* comprises regions A-E. Region C (membrane transport region) comprises four genes (*ctrA to D)* and region A (biosynthesis region) also comprises four genes (*synX to D).* The region C genes are separated from the region A genes by a 134 bp intergenic region which contains transcriptional start sites for both *ctrA* and *synX* preceded by promoter binding sequences. Regions C and A are divergently transcribed from the intergenic region.

The role of these genes in the serogroup B capsule synthesis pathway is shown in Fig. 1B. *synX* encodes either the *N*-acetyl-D-glucosamine-6-phosphate 2-epimerase that produces *N*-acetyl-D-mannosamine-6-phosphate or a specific phosphatase that converts *N*-acetyl-D-mannosamine-6-phosphate into *N*-acetyl-D-mannosamine [Swartley and Stephens (1994) *supra*]. *synB* encodes CMP-N-acetylneuraminic acid (NANA) synthetase [Edwards and Frosch (1992) *supra*]. *synC* encodes NANA synthetase [Ganguli et al. (1994) *supra*] and *synD* encodes the polysialyltransferase responsible for ($\alpha 2\rightarrow 8$)-linked polysialic acid chain polymerization and elongation [Frosch et al. (1991) *supra*].

The genetic structure of the capsule transport and biosynthetic regions was assessed with Southern analysis, PCR and DNA sequencing in strains from each of the other major meningococcal serogroups as shown in Figs. 1C and 1D. The strains of the sialic acid capsule-expressing serogroups (B, C, Y, W-135) were found to have a similar genetic organization consisting of the *ctrA* capsule transport gene linked by a short intergenic region to the oppositely transcribed biosynthetic genes *synX-synC.* Identical Southern hybridization patterns were obtained for *ctrA* (Fig. 1C), *synX, synB* and *synC;* identical PCR amplification products (Fig. 1D) were obtained for *ctrA, synX, synB* and *synC;* and similar nucleotide sequences were obtained for *ctrA-synX* intergenic region. These facts of identity established that *ctrA and synX-C* in serogroups C, Y, and W-135 *N. meningitidis were* homologues of the

corresponding genes in serogroup B meningococci. In contrast, *synD* [the serogroup B (α2→8)-linked capsule polysialyltransferase [Frosch et al. (1991) *supra*] was not detected in the serogroup C, Y and W-135 strains by Southern hybridization or PCR amplification using probes specific for *synD* of serogroup B (Fig. 1D).

Further, the nucleotide sequences of the 3' end of *synC* and the sequence downstream of *synC* were determined in serogroups C, Y, and W-135. The sequences of the 3' end of *synC* from serogroups B, C, Y, and W-135 were identical up to the last codon where the sequences then diverged (Fig. 2). The 5' ends of the downstream ORF's which encode the putative sialic acid capsule, polymerases (designated in serogroup B as *synD*, in serogroup C as *synE*, and in serogroups Y and W-135 as *synF*), were distinct (Fig. 2). In the serogroup Y and W-135 strains, the codon for the last amino acid in *synC* had been replaced by a different codon (creating a change from glutamine to serine). The nucleotide sequences downstream of *synC* were almost identical in serogroups Y and W-135 both in the intergenic region and in the first 800 bases of the 5'-end of the predicted capsule polymerase, but were distinct from serogroups B and C.

Thus, meningococci expressing serogroup B, C, Y, or W-135 sialic acid capsules have similar *synX-C* biosynthetic genes which are linked to *ctrA* of the capsule membrane transport operon. However, the genes encoding the sialic acid capsule polymerases in serogroups B, C, and Y/W-135 are different. Meningococci of serogroups Y and W-135 are almost identical in the 5'-end of this gene. These are closely related serogroups and simultaneous elaboration of both serogroup W-135 and Y capsular polysaccharides by a single strain of *N. meningitidis* has been reported [Brandt et al. (1980) *J. Gen. Microbiol.* **118**:39-43].

In contrast to the sialic acid serogroups, serogroup A meningococci contain a *ctrA* homologue but do not have a *ctrA-synX* intergenic region or the sialic acid biosynthetic homologues *synX-synD*. Serogroup A *ctrA* differs in nucleotide sequence and *ClaI* fragment size from the sequence and location of *ctrA* in the sialic acid capsule-expressing serogroups (Fig. 1C). Instead of exhibiting a 134 bp intergenic region separating *ctrA* from *synX* as found in all of the sialic acid producing serogroups (B, C, Y and W-135), the serogroup A *ctrA* gene is preceded by a 218 bp intergenic region. The serogroup A intergenic region separates *ctrA* from four novel co-transcribed open reading frames, which have been designated *orf1*,*orf2*, *orf3* and *orf4*. Since serogroup A does not produce a sialic acid

containing capsule, the capsule biosynthetic genes are different from those of serogroups B, C, Y and W-135. The serogroup A biosynthetic genes are only found in serogroup A and not in the other meningococcal serogroups. Southern and PCR analyses revealed that for a particular serogroup, the genes (e.g., *synD, synE, synF)* involved in alternative capsule polymerization were not present elsewhere in the chromosome (e.g., serogroup B strains contains *synD* but not *synE or synF* homologues.

The meningococcal capsule biosynthesis operon can be transformed *in vitro*. Meningococci are naturally competent for transformation. Conversion from one sialic acid expressing capsule serogroup to another was accomplished by homologous recombination of the sequences encoding the serogroup-specific capsule polymerase. Chromosomal DNA containing a Class I Tn916 insertion interrupting *synD* of the serogroup B strain NMB [Swartley et al. (1996) *J. Bacteriol.* **178**:4052-4059] was prepared and used to transform [Swartley et al. (1993) *Mol. Microbiol.* **10**:361-369] the prototype serogroup C, Y, and W-135 meningococcal strains. Tetracycline-resistant transformants were obtained at a frequency of between 1×10^{-5} and 1×10^{-7}/recipient. Acquisition of the Tn916 mutation and the adjacent *synD* sequence was confirmed by PCR and nucleotide sequence analysis of selected tetracycline-resistant transformants of these strains. Induced excision of the Tn*916* transposon insertion restores *synD* activity at a frequency of approximately 1×10^{-4}. Restoration of *synD* resulted in the expression of (α2→8)-linked polysialic acid capsule (Serogroup B) in an otherwise isogeneic serogroup C prototype strain.

The ability to transform a meningococcal capsule biosynthesis operon *in vitro* suggested an *in vivo* occurrence of such an event. The capsule biosynthesis and transport genes in serogroup B and serogroup C ET-5 complex strains from the Pacific Northwest outbreak were analyzed to determine if a transformation event involving the capsule biosynthesis genes produced the closely related serogroup B and C meningococcal strains recovered in the Oregon and Washington State outbreak. The analysis also included unlinked genes in two serogroup B and three serogroup C ET-5 complex strains (Table 1) recovered from this outbreak. These strains by ET-type (301), serotype (15), subtype (1.7,16), immunotype (L3,7,9), and PFGE type were identical; they differed only in the type of capsule produced.

TABLE 1

N. meningitidis isolates of the ET-5 complex recovered from patients with invasive meningococcal disease in Oregon in 1994

ID no.	Date of onset of illness	Sero-group	Serotype	Subtype	Immuno-type	ET type	PFGE type
B301#1 1070	06/21/94	B	15	1.7,16	L3,7,9	301	A
B301#2 1069	06/13/94	B	15	1.7,16	L3,7,9	301	A
C301#1 1205	11/19/94	C	15	1.7,16	L3,7,9	301	A
C301#2 1198	08/08/94	C	15	1.7,16	L3,7,9	301	A
C301#3 1204	10/29/94	C	15	1.7,16	L3,7,9	301	A

The capsule biosynthesis operon was analyzed in the different strains. By PCR and Southern hybridization profile, the strains showed similar *ctrA and synX-C* homologues, but the serogroup B ET-301 strains contained a *synD* homologue, whereas the serogroup C ET-301 strains contained a *synE* homologue. This observation was confirmed by determination of the nucleotide sequences of the intergenic region following *synC* as well as the sequences of the 5'-end of the downstream gene encoding the predicted polysialyltransferase. As shown in Fig. 3A, these regions were distinct in strain 1070 (serogroup B, ET-301) and 1205 (serogroup C, ET-301) isolates, exhibiting only 63% nucleotide identity. However, the nucleotide sequence of *synD* in the B301 strain was 99% identical to *synD* of the prototype serogroup B strain NMB; and in the C301 strain, *synE* was 99% identical to *synE* of the prototype serogroup C strain FAM18. Nucleotide sequences *of synX* and *synC* from strains 1070 and 1205 demonstrated 1% *(synX)* and 5% (*synC*) diversity (Figs. 3A and 3B1) suggesting that the polysialyltransferase gene and the entire *synX-D* biosynthetic operon had exchanged.

The extent of the recombinational event was determined by analyzing other operons. In contrast to the biosynthesis operon, the 5' nucleotide sequence of *ctrA* and the *ctrA-synX* intergenic region were identical in B-301 strains 1070 and 1069 and C-301 strains 1205, 1198 and 1204, but differed from other B, C, Y, and W-135 strains (Fig. 3B1). For example, the two B-301 and three C-301 strains contained the same *synX-ctrA* intergenic nucleotide sequence including an 8 bp deletion. In addition, the nucleotide sequence of two genes *(recA* [Zhou et al. (1992) *Mol. Microbiol.* 6:2135-2146] and *fkbp* [McAllister et al. (1993) *Mol. Microbiol.* **10**:13-23]) not linked to capsule expression were also identical in the B-301 and C-301 strains, but the sequence differed by up to 5% from other meningococcal strains (Fig. 3B2 and Fig. 3B3).

Thus, capsule switching of the epidemic serogroup B/C isolates was the result of substitution of the serogroup B *synD* polysialyltransferase with the serogroup C *synE* polysialyltransferase. Upstream of the polysialyltransferases, the recombinational event also included the conserved CMP-NANA biosynthesis genes, *synX-synC,* but did not extend to *ctrA* or the intergenic region separating *ctrA-synX,* and did not involve unlinked genes. The downstream recombinational exchange did not occur in *galE.* PCR studies using primers specific for the 3' end of *synC* and the 5' end of *galE* [Zhou et al. (1994) *J. Biol. Chem.* **269**:11162-11169] indicated that *synD/E* were downstream from *galE* by approximately 2 kb in the prototype serogroup B strain, NMB, in the prototype serogroup C strain, FAM18, and in each of the B-301 and C301 strains. However, PCR amplification of chromosomal DNA using internal *galE*-specific primers derived from the NMB *galE* sequence yielded a 900 bp product; but this product was not obtained with the serogroup C prototype strains FAM18, and two other non-ET-301 serogroup C strains (GA0078-ET-17, GA0290, ET-27).

Capsule switching in *N. meningitidis* can occur by gene conversion of the capsule polymerase and that this event occurs *in vivo.* Presumably, co-colonization of serogroup B and C strains in the human nasopharynx and genetic exchange of capsule biosynthesis genes by transformation and allelic-exchange is the event responsible for capsule switching. The high frequency (5-10%) of meningococci in the human nasopharynx of adults [Greenfield et al. (1971) *J. Infect. Dis.* **123**:67-73], which appears to increase in epidemic settings, may increase the likelihood of capsule switching. There are meningococcal strain collections which contain isolates with identical genetic markers (e.g., ET-type) but that express different

capsular polysaccharides. In addition to the meningococcal epidemic in the Pacific Northwest, recent cases in the Czech Republic and Canada [Kriz, P. and Musilek, M. *Abstracts of the Tenth International Pathogenic Neisseria Conference*, Zollinger, W.D., Frasch, C.E. and Deal, C.D. (eds.), Poster 174, p. 482, Baltimore, MD; Ashton, F.E. et al. (1996) *Abstracts of the Tenth International Pathogenic Neisseria Conference*, Zollinger, W.D., Frasch, C.E. and Deal, C.D. (eds.), Poster 148, p. 431, Baltimore, MD] of meningococcal disease caused by B and C strains with identical serotypes and ET types suggest that capsule switching may be common. The ability to switch capsules provides a selective advantage to meningococci, due to evasion of killing, opsonization or neutralization by pre-existing anticapsular antibody. Moreover, capsule switching also occurs in encapsulated *Streptococcus pneumoniae* and *Haemophilus influenzae* [Coffey, T.J. et al. (1991) *Mol. Microbiol.* 5:2255-2260; Kroll, J.S. and Moxon, E.R. (1990) *J. Bacteriol.* **172**:1374-1379].

The nucleotide sequence (SEQ ID NO:8) spanning the region between *ctrA* and *galE* in the encapsulated serogroup A *N. meningitidis* strain F8229 was determined using a combination of standard and single-specific-primer (SSP)-PCR. Primer LJ4, which anneals to sequence complementary to the 5' end of *ctrA* (Table 2) was used to begin "chromosome walking" 2.2 kilobases (kb) upstream of *ctrA* in strain F8229 by SSP-PCR. Next, primer SE33, designed to anneal to the 3' end of the 2.2 kb region, and primer GalE1, designed to anneal to sequence complementary to the 5' end of *galE*, were used to PCR amplify an additional 2.5 kb of intervening DNA. The double-stranded sequence of the 5064 bp stretch separating *ctrA* from *galE* in serogroup A *N. meningitidis* was determined from these products and confirmed by a combination of manual and automated DNA sequencing methods.

Computer analysis of the approximately 5 kb sequence (SEQ ID NO:8) indicated the presence of four ORFs transcribed in the opposite orientation with respect to *ctrA*. The first ORF (ORF1) was separated from *ctrA* by a 218 base pair (bp) intergenic region. ORF1 (nucleotides 479-1597) was 1119 nucleotides long and was predicted to encode a 372 amino acid protein (SEQ ID NO:9). ORF1 was separated by a single base from ORF2 (nucleotides 1599-3236), which was 1638 bp long, and was predicted to encode a 545 amino acid protein (SEQ ID NO:10). ORF2 was in turn separated by 72 bp from a 744 bp ORF, designated

TABLE 2

Primer Name	Nucleotide Sequence (5'→3')	SEQ ID NO:
LJ4	CCACCACCAAACAATACTGCCG	[SEQ ID NO:36]
SE33	GTCAACTCAGAAGATAAGAATTGG	[SEQ ID NO:37]
SE35	TCTCTTTTGTGATTCCGCTCC	[SEQ ID NO:38]
SE40	GAATAGCACTACATGCACTTCCC	[SEQ ID NO:39]
SE41	CAGGGCGAGTGCCAAAGACG	[SEQ ID NO:40]
SE46	GAAGCTGTAGCTGCAGGAACTG	[SEQ ID NO:41]
SE56	AATCATTTCAATATCTTCACAGCC	[SEQ ID NO:42]
SE57	TTACCTGAATTTGAGTTGAATGGC	[SEQ ID NO:43]
SE58	GTACCAATCAAAGGCGATATTGG	[SEQ ID NO:44]
SE61	CAAAGGAAGTTACTGTTGTCTGC	[SEQ ID NO:45]
SE63	TTCATATAACTTGCGGAAAAGATG	[SEQ ID NO:46]
JS102	GAGCCTATTCGAAATCAAAGCTG	[SEQ ID NO:47]
JS103	AGATACCATTAGTGCATCTATGAC	[SEQ ID NO:48]
JS104	CATGAAACTCAGCACAGATAGAAC	[SEQ ID NO:49]
JS105	GTTATTTAAATCTAGCCATGTGG	[SEQ ID NO:50]
galE1	CGTGGCAGGATATTGATGCTGG	[SEQ ID NO:51]

ORF3 (nucleotides 3309-4052), predicted to encode a 247 amino acid protein (SEQ ID NO:11). Finally, ORF3 was separated by a single nucleotide from an 864 bp ORF, designated ORF4 (nucleotides 4054-4917), which was predicted to encode a 287 amino acid protein (SEQ ID NO:12). The organization of ORFs located between *ctrA* and *galE* is presented schematically in Fig. 4.

In addition to the sequence derived from encapsulated wild-type strain F8229, the first 2330 bp of the *ctrA-galE* intervening region in the unencapsulated serogroup A variant strain F8239 was also sequenced. Comparison of the nucleotide sequences derived from F8239 and F8229 indicated that they were nearly identical (11 nucleotide differences [7 deletions or additions, 2 transversions, 2 transitions] over the entire 2.2 kb stretch). However, in strain F8239, ORF1 was only 744 nucleotides long (247 amino acid predicted protein). Computerized alignment of the putative amino acid translation of the F8239 and F8229 ORF sequences indicated that in F8239, ORF2 was prematurely truncated by a frame-shift mutation.

Nucleotide and predicted amino acid sequences of the putative ORFs were compared to the GenBank/EMBL and FA1090 gonococcal genome project database. ORF1 showed best homology (57.6% amino acid identity) with a cytoplasmic *E. coli* protein designated

NfrC. The 1131 bp *nfrC* gene encodes a 377 amino acid protein predicted to be a UDP-N-acetyl-D-glucosamine 2-epimerase [Kiino, D.R. et al. (1993) *J. Bacteriol.* 175:7074-7080]. ORF2 demonstrated limited nucleotide and amino acid sequence identity with two separate ORFs of unknown function, a 1125 bp open reading frame found downstream of *galE/rfbBCD* in serogroup B *N. meningitidis* (26.8% identity) and the 1632 bp *cpsY* of *Mycobacterium leprae* (37.7% identity). ORF3 and ORF4 did not exhibit significant nucleotide or amino acid homology with any genes or proteins in the databases. ORF1-4 were not present in the genomes of other meningococcal serogroups or *N. gonorrhoeae* by data base search, Southern hybridizations or PCR. It is proposed that ORF2 is the polymerase linking individual UDP-ManNAc monomers. The first biosynthetic step in the pathway is the production of UDP-ManNAc from UDP-NAc performed by the gene product of ORF1. ORF2 likely encodes the UDP-N-acetyl-mannosamine (α1→6) polymerase, and ORF3 and ORF4 proteins are believed involved in further modification and assembly of the serogroup A capsule.

The biosynthesis of the serogroup A capsule of *N. meningitidis* requires genes that are not found in other meningococcal serogroups. However, the general overall genomic organization of the capsule transport and biosynthesis regions of serogroup A meningococci and of the sialic acid containing capsular serogroups (B, C, Y and W-135) is similar. In all serogroups, the genes of the *ctr* capsule transport operon are preceded by an intergenic region which separates *ctrA-D* from an operon of divergently transcribed genes involved in capsule biosynthesis (SEQ ID NO:35; Fig. 5). These biosynthesis genes lie between *ctrA* and the gene encoding the UDP-glucose-4-epimerase (*galE*) necessary for LOS biosynthesis. Thus, differences in capsule composition between meningococcal serogroups are determined by proteins encoded in the distinct genetic cassettes located between *ctrA* and *galE*.

To determine whether ORF1-ORF4 were an operon, RT-PCR determinations were performed on whole cell RNA obtained from strain F8229. It was shown that ORF1-ORF4 are co-transcribed on the same mRNA message and therefore constitute an operon. The start site of transcription of the ORF1-ORF4 operon, as defined by primer extension (Fig. 6), was located within the 218 bp intergenic region separating *ctrA* and ORF1 (Fig. 7). The putative transcriptional start site was preceded by a putative σ-70-type promoter consensus sequence. The serogroup A *ctrA* transcriptional start site was also present in the 218 bp intergenic

region as shown by primer extension (Fig. 6). It was also preceded by a near consensus σ-70-type promoter that overlapped the ORF1 promoter.

To confirm the role of ORF1-ORF4 in serogroup A capsule expression, insertion mutations were created in each of the ORFs in the wild-type encapsulated strain F8229. Strains F8229ORF1Ω, F8229ORF2Ω, F8229ORF3Ω, and F8229ORF4Ω were created by Ω-spectinomycin insertional mutagenesis of specific ORFs in wild-type encapsulated serogroup A strain F8229. The results of colony immunoblots using labeled Serogroup A-specific antibody demonstrated that polar mutagenesis of all four ORFs in wild-type strain F8229 resulted in a reduction or loss of encapsulation. These data were confirmed using a quantitative capsule whole cell ELISA (Table 3).

Attempts to create non-polar interruptions of ORF1 and ORF2 by integrating an *aphaA-3* cassette into the same unique sites used for the Ω-cassette mutagenesis resulted only in the integration of this fragment into ORF2. Like the polar Ω-spectinomycin knock-out mutants, the non-polar interruption of ORF2 also resulted in a loss of group A capsule expression, as visualized by colony immunoblots and whole cell ELISA (strain F8229ORF2*aph3*, Table 3).

TABLE 3

Strain	Mean A_{405}	SD	% reduction vs wild-type
F8229	0.939	0.016	N.A.
F8239	0.000	0.000	100%
F8229-ORF1Ω	0.000	0.000	100%
F8229-ORF2Ω	0.000	0.000	100%
F8229-ORF2aphA-3	0.000	0.000	100%
F8229-ORF3Ω	0.000	0.000	100%
F8229-ORF4Ω	0.101	0.007	89%

The invention also provides a vaccine based on capsule polysaccharide structure and a method for vaccinating a population at risk during an epidemic outbreak. Further, the

invention provides for epidemiologic investigations of disease due to encapsulated bacteria. For example, meningococci of different serogroups recovered during epidemic outbreaks or from cases of endemic disease can be identical in their expression of other virulence factors (e.g., outer membrane proteins) but express different capsular polysaccharides. Meningococcal capsule switching appears to occur among sialic acid-expressing strains (Serogroups B, C, Y and W) by allelic replacement of the sialic acid capsule polymerase.

Table 4 provides a list of meningococcal strains in which capsule switching has been observed. Strains of all serogroups, i.e., A, B, C, Y and W-135, have been transformed and subject to gene, or operon, recombination.

TABLE 4

Meningococcal Strains Exhibiting Capsule Switching Recombination.

Strain	Phenotype
NMB-43	Mutant derivative of parental strain NMB (clinically isolated serogroup B *Neisseria meningitidis*). Contains Class I Tn*916* insertion in the *synD* polysialyltransferase gene inactivating group B capsule production. Mutation has been mobilized into prototype strains of other serogroups as described below.
NMB-M7	Mutant derivative of parental strain NMB. Contains Class I Tn*916* insertion in the *synX* capsule biosynthesis gene inactivating group B capsule production. Mutation has been mobilized into prototype strains of other serogroups as described below.
Fam18-43	Serogroup C prototype strain transformed with 43 mutation from NMB-43.
Fam18-M7	Serogroup C prototype strain transformed with M7 mutation from NMB-M7.
1205	Serogroup C, ET301 strain isolated from Oregon outbreak.
1205-43	Serogroup C, ET301 strain isolated from Oregon outbreak transformed with the 43 mutation from NMB-43.
1205-43CC	Capsule conversion derivative of strain 1205-43 in which the transposon insertion has precisely excised from the transformed *synD* gene resulting in the production of serogroup B capsule.

1205-M7	Serogroup C, ET301 strain isolated from Oregon outbreak transformed with the M7 mutation from NMB-M7.
1198	Serogroup C, ET301 strain isolated from Oregon outbreak.
1204	Serogroup C, ET301 strain isolated from Oregon outbreak.
F8229	Serogroup A prototype strain obtained from the CDC and originally isolated on the African Continent. Encapsulated.
F8239	Unencapsulated variant of the same serogroup A prototype strain.
F8239-43	Serogroup A prototype strain obtained from the CDC and originally isolated on the African Continent transformed with the 43 mutation from NMB-43.
F8239-M7	Serogroup A prototype strain obtained from the CDC and originally isolated on the African Continent transformed with the M7 mutation from NMB-M7.
GA0929	Serogroup Y prototype strain isolated as part of the Metropolitan Atlanta Active Surveillance Project. Encapsulated.
GA0929-43	Serogroup Y prototype strain transformed with the 43 mutation from NMB-43.
GA0929-M7	Serogroup Y prototype strain transformed with the M7 mutation from NMB-M7.
GA1002	Serogroup W-135 prototype strain isolated as part of the Metropolitan Atlanta Active Surveillance Project. Encapsulated.
GA1002-43	Serogroup W-135 prototype strain transformed with the 43 mutation from NMB-43.
GA1002-M7	Serogroup W-135 prototype strain transformed with the M7 mutation from NMB-M7.

This invention embodies a general strategy by which meningococci and other encapsulated bacteria capable of causing epidemic outbreaks or endemic disease escape vaccine-induced or natural protective immunity. In view of this discovery, this invention provides multivalent vaccines effective against all major capsular serogroups, which vaccines are needed to control epidemics and possibly endemic disease.

Techniques are available for the generation of stable insertion mutations in *N. meningitidis* and other *Neisseria* species. Stephens and co-workers has described Tn916

mutagenesis of different neisserial species [Stephens et al. (1991) *Infect. Immun.* 59:4097-4102; Stephens et al. (1994) *Infect. Immun.* 62:2947-2952; Kathariou et al. (1990) *Mol. Microbiol.* 4:729-735]. Two types of insertion mutations occur: class I insertions appear to have an intact Tn916 element resulting from transposition of the transposon and class II insertions are characterized by deletion of part of the transposon with maintenance of the *tet*M element which confers tetracycline resistance. Insertions can be characterized in part with analysis of HaeIII-digested DNA in that Tn916 has no *Hae*III sites, and the portion of the genome into which the transposon or tetracycline-resistance determining region has inserted by subcloning a HaeIII fragment with selection for antibiotic resistance. Flanking sequences can be used for sequence determination and/or for use in probe or primer for the isolation of the wild-type counterpart gene from the parental strain. Stable mutations can be generated, including, but not limited to, deletion mutations, insertion mutations or multiple point mutations, and this may be accomplished by techniques including but not limited to oligonucleotide site-directed mutagenesis, polymerase chain reaction mutagenesis techniques, restriction endonuclease cutting and religation with or without insertion of heterologous DNA as appropriate for the type of mutation being created, as well known to one of ordinary skill in the art. The skilled artisan is capable of generating such alternate mutants using ordinary skill in the art; in particular, the DNA sequence information provided herein (e.g., serogroup C *synE* (SEQ ID NO:2), serogroup Y *synF* (SEQ ID NO:3), serogroup W-135 *synF* (SEQ ID NO:4) and serogroup A *orf1-orf4* (SEQ ID NO:8) can be employed in mutagenic strategies. The sequence information provided can be used to produce multiple mutations. It is preferred that where a transposon is used, the resulting mutation itself is not an insertion which is further transposable.

The skilled artisan recognizes that other neisserial (and certain *H. influenzae*) strains can express a non-isogeneic serogroup capsular polysaccharide as expressed by the recombinant characteristics of *N. meningitidis* B-301 strains 1070 and 1069, for example. The distinguishing characteristics of these recombinant strains (e.g., B-301 1070 and 1069) are (a) the presence of a capsular polysaccharide enzyme gene specific to serogroup C *N. meningitidis* strains (C *synE*) encoding (α2→8)-linked polysialyltransferase in an otherwise isogeneic (serogroup B) capsule biosynthesis operon and (b) immunological resistance to a vaccine based on solely serogroup B capsule polysaccharide epitopes (e.g., (α2→8)-linked

polysialic acids). A recombinant strain of *N. meningitidis* can be identified not only by the presence of a gene encoding a capsular polysaccharide of a different serotype, but also by specific binding to a monoclonal antibody to a capsular polysaccharide of a non-isogeneic serogroup. In view of the similarity of the basic structures of capsular polysaccharide molecules of the meningococci, gonococci and certain *H. influenzae* strains, the skilled artisan understands that an antibody, particularly a monoclonal antibody which is specific for a particular epitope, directed to a particular capsular polysaccharide of a meningococcal specific serogroup strain can be used to screen other encapsulated bacterial strains for the presence of the epitopes recognized by that (monoclonal) antibody.

A polynucleotide or fragment thereof is substantially homologous (or substantially similar) to another polynucleotide if, when optimally aligned (with appropriate nucleotide insertions or deletions) with another polynucleotide, there is nucleotide sequence identity for approximately 80% of the nucleotide bases, usually approximately 90%, more preferably about 95% to 100% of the nucleotide bases.

Alternatively, substantial homology (or similarity) exists when a polynucleotide or fragment thereof will hybridize to another polynucleotide under selective or stringent hybridization conditions. Selectivity of hybridization exists under stringent hybridization conditions which allow one to distinguish the target polynucleotide of interest from other polynucleotides. Typically, selective hybridization will occur when there is approximately 75% similarity over a stretch of about 14 nucleotides, preferably approximately 80% similarity, more preferably approximately 85% similarity, and most preferably approximately 90% similarity. See Kanehisa (1984) *Nucl. Acids Res.* (12:203-213. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will often be over a stretch of about 17 to 20 nucleotides, preferably 21 to 25 nucleotides, more preferably 26 to 35 nucleotides, and more preferably about 36 or more nucleotides.

The hybridization of polynucleotides is affected by such conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing polynucleotides, as will be readily appreciated by those skilled in the art. Stringent temperature conditions will generally include temperatures in excess of 30°C, typically in excess of 37°C, and preferably in excess of 45°C. Stringent salt conditions will

ordinarily be less than 1 M, typically less than 500 mM, and preferably less than 200 mM. However, the combination of parameters is much more important than the measure of any single parameter [Wetmur and Davidson (1968) *J. Mol. Biol.* 31, 349-370].

An isolated or substantially pure polynucleotide is a polynucleotide which is substantially separated from other polynucleotide sequences which naturally accompany a native sequence. The term embraces a polynucleotide sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates, chemically synthesized analogues and analogues biologically synthesized by heterologous systems.

A polynucleotide is said to encode a polypeptide if, in its native state or when manipulated by methods known to those skilled in the art, it can be transcribed and/or translated to produce the polypeptide of a fragment thereof. The anti-sense strand of such a polynucleotide is also said to encode the sequence.

A nucleotide sequence is operably linked when it is placed into a functional relationship with another nucleotide sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression. Generally, operably linked means that the sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. However, it is well known that certain genetic elements, such as enhancers, may be operably linked even at a distance, i.e., even if not contiguous.

The term recombinant polynucleotide refers to a polynucleotide which is made by the combination of two otherwise separated segments of sequence accomplished by the artificial manipulation of isolated segments of polynucleotides by genetic engineering techniques or by chemical synthesis. In so doing one may join together polynucleotide segments of desired functions to generate a desired combination of functions.

Polynucleotide probes include an isolated polynucleotide (or oligonucleotide) attached to a label or reporter molecule and may be used to identify and isolate hybridizing, homologous coding sequences. Probes comprising synthetic oligonucleotides or other polynucleotides may be derived from naturally occurring or recombinant single or double stranded nucleic acids or be chemically synthesized. Polynucleotide or oligonucleotide

probes may be labeled by any of the methods known in the art, e.g., random hexamer labeling, nick translation, or the Klenow fill-in reaction.

Large amounts of the polynucleotides may be produced by replication in a suitable host cell. Natural or synthetic DNA fragments coding for a proteinase or a fragment thereof will be incorporated into recombinant polynucleotide constructs, typically DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the construct will be suitable for replication in a unicellular host, such as yeast or bacteria, but a multicellular eukaryotic host may also be appropriate, with or without integration within the genome of the host cells. Commonly used prokaryotic hosts include strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or *Pseudomonas* may also be used. Mammalian or other eukaryotic host cells include yeast, filamentous fungi, plant, insect, amphibian and avian species. Such factors as ease of manipulation, ability to appropriately glycosylate expressed proteins, degree and control of protein expression, ease of purification of expressed proteins away from cellular contaminants or other factors may determine the choice of the host cell.

The polynucleotides or oligonucleotides may also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage and Caruthers (1981) *Tetra. Letts.*, 22: 1859-1862 or the triester method according to Matteuci et al. (1981) *J. Am. Chem. Soc.* 103: 3185, and may be performed on commercial automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

DNA constructs prepared for introduction into a prokaryotic or eukaryotic host will typically comprise a replication system (i.e. vector) recognized by the host, including the intended DNA fragment encoding the desired polypeptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide-encoding segment. Expression systems (expression vectors) may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator

sequences, and mRNA stabilizing sequences. Signal peptides may also be included where appropriate from secreted polypeptides of the same or related species, which allow the protein to cross and/or lodge in cell membranes or be secreted from the cell.

An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host. Examples of workable combinations of cell lines and expression vectors are described in Sambrook et al. (1989) vide infra; Ausubel et al. (Eds.) (1987) *Current Protocols in Molecular Biology*, Greene Publishing and Wiley Interscience, New York; and Metzger et al. (1988) *Nature*, 334: 31-36. Many useful vectors for expression in bacteria, yeast, mammalian, insect, plant or other cells are well known in the art and may be obtained such vendors as Stratagene, New England Biolabs, Promega Biotech, and others. In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made. For appropriate enhancer and other expression control sequences, see also *Enhancers and Eukaryotic Gene Expression*, Cold Spring Harbor Press, N.Y. (1983). While such expression vectors may replicate autonomously, they may less preferably replicate by being inserted into the genome of the host cell.

Expression and cloning vectors will likely contain a selectable marker, that is, a gene encoding a protein necessary for the survival or growth of a host cell transformed with the vector. Although such a marker gene may be carried on another polynucleotide sequence co-introduced into the host cell, it is most often contained on the cloning vector. Only those host cells into which the marker gene has been introduced will survive and/or grow under selective conditions. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxic substances, e.g., ampicillin, neomycin, methotrexate, etc.; (b) complement auxotrophic deficiencies; or (c) supply critical nutrients not available from complex media. The choice of the proper selectable marker will depend on the host cell; appropriate markers for different hosts are known in the art.

The recombinant vectors containing the capsule polysaccharide biosynthetic gene (or mutant gene) sequence of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation; transformation or transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and transfection or infection (where the vector is an infectious agent, such as a viral or retroviral genome). The choice of such means will

often depend on the host cell. Large quantities of the polynucleotides and polypeptides of the present invention may be prepared by transforming suitable prokaryotic or eukaryotic host cells with capsular polysaccharide-related polynucleotides of the present invention in compatible vectors or other expression vehicles and culturing such transformed host cells under conditions suitable to attain expression of the desired capsular polysaccharide structure. The derivative polysaccharide may then be recovered from the host cell and purified. For example, it may be possible to create recombinant polysialyltransferases that could be over-expressed, purified, and used *in vitro* reactions to create capsular polysaccharide materials of substantial purity. Substantially pure capsular polysaccharides can be used as hybridization probes or in the preparation of vaccines.

When it is desired to eliminate leader sequences and precursor sequences at the 5' side of the coding sequence, a combination of restriction endonuclease cutting and site-directed mutagenesis via PCR using an oligonucleotide containing a desired restriction site for cloning (one not present in coding sequence), a ribosome binding site, a translation initiation codon (ATG) and the codons for the first amino acids of the mature protein. The oligonucleotide for site-directed mutagenesis at the 3' end of the coding sequence includes nucleotides encoding the carboxyterminal amino acids of the protein, a translation termination codon (TAA, TGA or TAG), and a second suitable restriction endonuclease recognition site not present in the remainder of the DNA sequence to be inserted into the expression vector. The site-directed mutagenesis strategy is similar to that of Boone et al. (1990) *Proc. Natl. Acad. Sci. USA* 87: 2800-2804, as modified for use with PCR.

In another embodiment, polyclonal and/or monoclonal antibodies capable of specifically binding to a particular serogroup capsular polysaccharide or fragments thereof are provided. The term antibody is used to refer both to a homogenous molecular entity and a mixture such as a serum product made up of a plurality of different molecular entities. Monoclonal or polyclonal antibodies, preferably monoclonal, specifically reacting with capsular polysaccharide of a particular serogroup of interest may be made by methods known in the art. See, e.g., Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratories; Goding (1986) *Monoclonal Antibodies: Principles and Practice*, 2d ed., Academic Press, New York. Also, recombinant immunoglobulins may be produced by methods known in the art, including but not limited to the methods described in U.S. Patent

No. 4,816,567, incorporated by reference herein. Monoclonal antibodies with affinities of 10^8 M^{-1}, preferably 10^9 to 10^{10} or more are preferred.

Antibodies generated against a specific serogroup capsular polysaccharide of interest are useful, for example, as probes for screening DNA expression libraries or for detecting the presence of neisserial strains in a test sample. Antigens can be synthesized and conjugated to a suitable carrier protein (e.g., bovine serum albumin or keyhole limpet hemocyanin) for use in vaccines or in raising specific antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or noncovalently, a substance which provides a detectable signal. Suitable labels include but are not limited to radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. United States Patents describing the use of such labels include but are not limited to Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

Antibodies specific for a particular serogroup capsular polysaccharide and capable of inhibiting adherence of neisserial and/or hemophilus cells expressing the particular capsular polysaccharide to host tissue are be useful in preventing disease resulting from neisserial and/or hemophilus infection. Such antibodies can be obtained by the methods described above.

Compositions and immunogenic preparations including vaccine compositions comprising substantially purified serogroup-specific capsular polysaccharides and a suitable carrier therefor are provided. Alternatively, antigens can be synthesized and conjugated to a suitable carrier protein (e.g., bovine serum albumin or keyhole limpet hemocyanin) for use in vaccines or in raising antibody specific for capsular polysaccharide-expressing neisserial and/or *H. influenzae* strains. Immunogenic compositions are those which result in specific antibody production when injected into a human or an animal. Such immunogenic compositions are useful, for example, in immunizing a humans, against infection by neisserial and hemophilus pathogenic strains. The immunogenic preparations comprise an immunogenic amount of, as specifically exemplified, at least one serogroup-specific capsular polysaccharide preparation derived from one serogroup strain of *N. meningitidis* and a suitable carrier. Alternatively, the immunogenic composition can comprise cells of at least one of the specifically exemplified recombinant *N. meningitidis* strains and a suitable carrier.

It is understood by one of ordinary skill in the art that other, functionally equivalent, recombinant strains of *N. meningitidis*, for example, B-301 strain 1070, can be produced by the introduction of the cloned DNA containing the insertion mutations responsible for a C serogroup characteristic. It is also within the scope of the present invention and readily within the grasp of the ordinary skilled artisan to generate other types of genetically stable mutations in the capsular polysaccharide enzyme genes of *N. meningitidis* and/or *N. gonorrhoeae* or *H. influenzae*. Such immunogenic compositions (or vaccines) are useful, for example, in immunizing an animal, especially humans, against neisserial disease resulting from infection by pathogenic neisserial species, particularly *Neisseria meningitidis* and *Neisseria gonorrhoeae*. Such immunogenic compositions can also elicit the production of antibodies which will cross react with capsular polysaccharides of, for example, *Hemophilus influenzae* strains expressing epitopes in common with those of the starting *N. meningitidis* strain(s). The immunogenic preparations comprise an immunogenic amount of an isogeneic or non-isogeneic serogroup capsular polysaccharide from a strain of *N. meningitidis* or *N. gonorrhoeae*, or an immunogenic fragment thereof, or of cells of one or more strains of Neisseria which express a specific serogroup capsular polysaccharide. Such immunogenic compositions advantageously further comprise capsular polysaccharides or neisserial cells of two or more other serological types, including, but not limited to, any known to the art, among which are serogroups A, B, C, D, E, H, I, K, L, W-135, X Y and Z [Apicella, M. (1995) *Neisseria meningitidis*, in Principles and Practice of Infectious Disease (4th edition), Eds. G.L. Mandell, J.E. Bennett and R. Dolin, Churchill Livingstone Inc., p. 1896]. It is understood that where whole cells are formulated into the immunogenic composition, the cells are preferably inactivated, especially if the cells are of a virulent strain. Such immunogenic compositions may comprise one or more additional capsular polysaccharide preparations, or another protein or other immunogenic cellular component. By "immunogenic amount" is meant an amount capable of eliciting the production of antibodies directed against neisserial capsular polysaccharides, including but not limited to those of exemplified *N. meningitidis* in an animal or human to which the vaccine or immunogenic composition has been administered.

Immunogenic carriers may be used to enhance the immunogenicity of the capsular polysaccharides. Such carriers include but are not limited to proteins and polysaccharides,

liposomes, and bacterial cells and membranes. Protein carriers may be joined to the capsular polysaccharide molecules to form fusion proteins by recombinant or synthetic means or by chemical coupling. Useful carriers and means of coupling such carriers to polypeptide antigens are known in the art. The art knows how to administer immunogenic compositions so as to generate protective immunity on the mucosal surfaces of the upper respiratory system, especially the mucosal epithelium of the nasopharynx, where immunity specific for *N. meningitidis* and for the remainder of the respiratory system, particularly for *H. influenzae*, and for the epithelial surfaces of the genito-urinary tract, particularly for *N. gonorrhoeae*, is most helpful.

The immunogenic compositions may be formulated by any of the means known in the art. Such vaccines are typically prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also, for example, be emulsified, or the protein encapsulated in liposomes.

The active immunogenic ingredients are often mixed with excipients or carriers which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients include but are not limited to water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. The concentration of the immunogenic polypeptide in injectable formulations is usually in the range of 0.2 to 5 mg/ml.

In addition, if desired, the vaccines may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants which may be effective include but are not limited to: aluminum hydroxide; N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP); N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP); N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE); and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogen resulting from administration of the immunogen in vaccines which are also comprised of the various

adjuvants. Such additional formulations and modes of administration as are known in the art may also be used.

Serogroup-specific capsular polysaccharides and cells producing capsular polysaccharides and/or fragments thereof may be formulated into immunogenic compositions as neutral or salt forms. Preferably, when cells are used they are of avirulent strains, or the cells are killed before use. Pharmaceutically acceptable salts include but are not limited to the acid addition salts (formed with free amino groups of the peptide) which are formed with inorganic acids, e.g., hydrochloric acid or phosphoric acids; and organic acids, e.g., acetic, oxalic, tartaric, or maleic acid. Salts formed with the free carboxyl groups may also be derived from inorganic bases, e.g., sodium, potassium, ammonium, calcium, or ferric hydroxides, and organic bases, e.g., isopropylamine, trimethylamine, 2-ethylamino-ethanol, histidine, and procaine.

The immunogenic capsular polysaccharide preparations (or peptide antigens related thereto) compositions are administered in a manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective. The quantity to be administered, which is generally in the range of about 100 to 1,000 μg of protein or polysaccharide per dose, more generally in the range of about 5 to 500 μg of protein per dose, depends on the subject to be treated, the capacity of the individual's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of the active ingredient required to be administered may depend on the judgment of the physician and may be peculiar to each individual, but such a determination is within the skill of such a practitioner.

The vaccine or other immunogenic composition may be given in a single dose or multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may include 1 to 10 or more separate doses, followed by other doses administered at subsequent time intervals as required to maintain and or reinforce the immune response, e.g., at 1 to 4 months for a second dose, and if needed, a subsequent dose(s) after several months.

Except as noted hereafter, standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are

those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook et al. (1989) *Molecular Cloning*, Second Edition, Cold Spring Harbor Laboratory, Plainview, New York; Maniatis et al. (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Plainview, New York; Wu (ed.) (1993) *Meth. Enzymol.* 218, Part I; Wu (ed.) (1979) *Meth Enzymol.* 68; Wu et al. (eds.) (1983) *Meth. Enzymol.* 100 and 101; Grossman and Moldave (eds.) *Meth. Enzymol.* 65; Miller (ed.) (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Old and Primrose (1981) *Principles of Gene Manipulation*, University of California Press, Berkeley; Schleif and Wensink (1982) *Practical Methods in Molecular Biology*; Glover (ed.) (1985) *DNA Cloning* Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) *Nucleic Acid Hybridization*, IRL Press, Oxford, UK; and Setlow and Hollaender (1979) *Genetic Engineering: Principles and Methods*, Vols. 1-4, Plenum Press, New York. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

All publications, patent applications and patents cited herein are incorporated by reference to the same extent as if each individual publication or patent were specifically and individually indicated to be incorporated by reference.

The foregoing discussion and the following examples are provided for illustrative purposes, and they are not intended to limit the scope of the invention as claimed herein. Modifications and variations which may occur to one of ordinary skill in the art are within the intended scope of this invention.

EXAMPLES

Example 1. Bacterial Strains

Forty serogroup B and C ET-5 complex meningococcal isolates recovered from Oregon, Washington State and California in 1994 and 1995 were used in these studies. In addition, meningococcal strains GA0078 (serogroup C GA0290[C]), NMB (B), C114 (B), M986 (B), 2996, (B), KB (B), 269B (B), FAM18 (C), 6083 (W-135), GA0929 (Y), F8229 (A), F8239 (A), NM-44/76 (B), GA1002 (W-135), *N. gonorrhoeae* strain FA19; and *N.*

lactamica and other commensal *Neisseria* spp. were also used [see Swartley et al. (1994) *J. Bacteriol.* 176, 1530-1534 and McAllister et al. (1993) *Mol. Microbiol.* 10, 13-23].

Serogroup A meningococcal strains F8229 and F8239 were originally isolated during an outbreak in Nairobi, Kenya in 1989 and were provided by the Centers for Disease Control and Prevention, Atlanta, Georgia. Strain F8229 (CDC #1750) is encapsulated and was clinically isolated from the cerebrospinal fluid of a patient. Strain F8239 (CDC #16N3) is an unencapsulated variant originally isolated as a serogroup A strain from the pharnyx of an asymptomatic carrier. These strains belong to clonal group III-1 and are closely related to strains that have caused recurrent epidemics in Saudi Arabia, Chad, Ethiopia and other parts of Africa. F8227ORF1Ω, F8229OF2Ω, F8229ORF2*apha3*, F8229ORF3Ω, and F8229ORF4Ω are serogroup A mutants created through insertional mutagenesis.

Meningococcal strain NMB (CDC #8201085) is a serogroup B (NT:P1.2,5:L3,7.9) strain originally isolated from the cerebrospinal fluid of a patient with meningococcal meningitis in Pennsylvania in 1982 [Stephens, D.S. et al. (1991) *Infect. Immun.* 59:4097-4102]. *Escherichia coli* strain αInvF' (Invitrogen) was used as the host strain for all cloned PCR products and recombinant plasmids created during these studies. Plasmid ρHP45 [Prentki, P. and Krisch, H.M. (1984) *Gene* 29:303-313] was the source of the spectinomycin resistant Ω-fragments used for polar gene mutagenesis and plasmid pUC18K [Menard, R. et al. (1993) *J. Bacteriol.* 175:5899-5906] was the source of the *apha-3* kanamycin resistance cassette used for the non-polar mutagenesis.

Example 2. Growth Conditions

Meningococcal strains were grown on GC base agar (Difco) or in GC broth (38) at 37°C with 3.5% CO_2. Minimal media with an without supplements were prepared as described previously [Swartley et al. (1994) *J. Bacteriol.* 176:1530-1534]. *E. coli* strains were grown on Luria-Bertani agar plates (Bethesda Research Laboratories) or in Luria-Bertani broth at 37°C. *E. coli* strain harboring putative *lacZ* transcriptional reporter gene constructs were screened on MacConkey agar plates (Difco). Antibiotics were used at the following concentrations: tetracycline (5 μg/ml), spectinomycin (100 μg/ml), kanamycin (60 mg/ml), and ampicillin (100 mg/ml).

Example 3. Molecular Epidemiology

Multiple enzyme electrophoretic (ET) typing was carried out according to the protocol described in Reeves et al. (1995), *Emerging Infect. Dis.* **2**:53-54, and pulsed field gel electrophoresis (PFGE) was performed as described in Bygraves et al. (1992) *J. Gen. Microbiol.* **138**:523-531. Specific enzyme types (e.g., ET-301) were designated by the Centers for Disease Control Meningococcal Reference Laboratory. Serotyping of meningococcal strains was done as described in Wedege et al. (1990) *J. Med. Microbiol.* **31**:195-201, with the following modifications: Meningococci were grown on brain heart infusion agar (BHI) (Difco) supplemented with 1% horse serum (Gibco), a higher concentration of cells (cell density 1.0 at OD_{600}), different blocking buffer (PBS + 0.1% Tween-20) and shorter primary antibody incubation (2.5 h).

Example 4. Transposon Mutagenesis

Tn916 is introduced into a strain of *N. meningitidis* of known serogroup by transformation as described [Kathariou et al. (1990) *Mol. Microbiol.* **4**:729-735], and the presence of the transposon is selected in solid medium with tetracycline. Preferably, the mutants isolated are the result of Class I insertions as described hereinabove.

The genetic stability during growth and laboratory passage for each Tn916 insertion mutant strain was tested. Only mutants having the phenotype of drug resistance and the presence of a non-isogeneic capsular polysaccharide gene as revealed by nucleotide sequence analysis were selected. Expression of a non-isogeneic serogroup marker is the result of homologous recombination via the DNA flanking the Tn916-derived portion of the DNA transformed into the parental strain.

Example 5. Capsular Polysaccharide Preparations

Meningococcal capsular polysaccharides are prepared according to Gotschlich et al. (1969) *J. Exp. Med.* **129**:1349-1365. Methods are disclosed for the preparation and analyses of immunological properties of serogroup A, B and C meningococcal polysaccharides.

Example 6. SDS Page Analysis

Tricine-SDS polyacrylamide gels (14% acrylamide) were prepared [Schagger and von Jagow (1987) *Anal. Biochem.* **166**:368-379] using the mini-Protean II apparatus (BioRad, Hercules, CA). Each sample is heated to 100°C for four minutes before loading. About 125 ng total protein is loaded per lane. The sample is electrophoresed at 30 V through the stacking gel and at 95 V through the separating gel. Prestained low molecular weight markers (Boehringer Mannheim, Indianapolis, IN) were used. Bands were visualized using the silver staining method as described in Hitchcock and Brown (1983) *supra*.

Example 7. Creation of Intergenic Region *lacZ* transcriptional reporter gene constructs

A 250-bp product containing the entire 134-bp intergenic region was PCR amplified and the produce was cloned in both orientations into the PCR product cloning vector pCR2000, using the TA PCR product cloning system (Invitrogen), thereby creating plasmids pCRINT1 and pCRINT2. The cloned intergenic region was then liberated from pCRINT1 and pCRINT2 with *Kpn*I and cloned into *Kpn*I-linearized, shrimp alkaline phosphatase (United States Biochemicals)-treated pEU730, a low-copy-number, promoterless, *lacZ* transcriptional fusion vector [Froehlich et al. (1991) *Gene* **108**:99-101]. The ligations were then transformed into *E. coli* MC4100 and plated on selective MacConkey agar. Strain MC4100 was used because its lactose utilization operon has been deleted and it forms white colonies on MacConkey media. We screened for transcriptionally active spectinomycin-resistant transformants (red colonies on MacConkey agar), indicating that we had cloned the *ctrA* promoter and the *synX* promoter of the intergenic region behind the *lacZ* gene of pEU730, thereby creating the target plasmids pEU730C and pEU730S, respectively. The promoter activities of these clones were measured by β-galactosidase assays.

Example 8. β-Galactosidase assays.

To investigate the possible promoter activities of cloned intergenic region constructs, we performed β-galactosidase assays with *E. coli* [Sambrook et al. (1989) supra]. Briefly, *E. coli* MC4100 strains harboring test and control constructs were grown to mid-log phase in complete liquid media. The cells were then pelleted and resuspended in a salt solution (1 liter, 5 x recipe: 64 g of $Na_2HPO_4 \cdot 7H_2O$, 15 g of KH_2PO_4, 2.5 g of NaCl, 5.0 g of NH_4Cl) and

the A_{600} was recorded. The cells were diluted in Z buffer (0.06 M Na_2HPO_4, 0.04 M NaH_2PO_4, 0.01 M KCl, 0.001 M $MgSO_4 \cdot 7H_2O$, 0.05 M β-mercaptoethanol [pH 7.0]), containing 0.1% SDS and chloroform. The diluted cells were then vortexed briefly, incubated at 28°C for 10 minutes, and then vortexed again. 0.2 ml ONPG (*O*-nitrophenyl-β-D-galactopyranoside) solution (4 mg of ONPG per mg in the aforementioned salt solution) was added to the lysed cells, and the time until a yellow color developed was measured. The reaction was then terminated by the addition of 1 M Na_2CO_3. The A_{420} and the A_{550} of the stopped reaction mixture were recorded, and Miller units were then calculated by the following formula: 1,000 x [A_{420} - (1.75 x A_{550})]/time in minutes x volume of cells used in milliliters x A_{600}.

Example 9: DNA Sequencing

For determination of the sequence flanking the Tn916-derived insertion, the fragment of DNA comprising the insertion is cloned into a suitable plasmid vector, for example, after *Hae*III digestion of chromosomal DNA. Double-stranded DNA was subcloned and sequenced by the dideoxy chain termination method [Sanger et al. (1977) *Proc. Natl. Acad. Sci. USA* 74:5463-5467], for example, using sequencing kits purchased from United States Biochemical Corporation (Cleveland, OH). Oligonucleotide primers for sequencing reactions are synthesized by the phosphoramidite method with an Applied Biosystems model 394 automated DNA synthesizer (Applied Biosystems, Foster City, CA), purified by PAGE and desalted on Sep-Pak (Millipore Corp., Beverly, MA) using standard protocols.

Example 10: Analytical Methods

The colony immunoblot screening was performed as described by Kahler et al. (1996) *J. Bacteriol.* 178:1265-1273. PCR, Southern DNA hybridization and DNA sequencing techniques were performed as previously described [Swartley et al. (1993) *Mol. Microbiol.* 10:361-369]. Automated sequencing using an ABI model 377 automated DNA sequencing system (Applied Biosystems, Foster City, CA) was performed on some PCR templates. Oligonucleotide primers used for PCR, sequencing and construction of Southern probes were:

5'*ctrA*: 5'GTGTGGAAGTTTAATTGTAGGATG-3' [SEQ ID NO:13;
3' *ctrA*: 5'-CCACCACCAAACAATACTGCCG-3' [SEQ ID NO:14];
5' *synX*: 5'-GCAATACCATTACGTTTATCTCTC-3' [SEQ ID NO:151];
3'*synX*: 5'-GTTTCAGGATTGTTGATTACTTCAGC-3' [SEQ ID NO:16];
5'*synB*: 5'-GTCCTACGCCCTGCAGAGCTGG-3' [SEQ ID NO:17];
3' *synB:* 5'-CATTAGGCCTAAATGCCTGAGG-3' [SEQ ID NO:18];
5' *synC*: 5'-GCTGAAGTTGTTAAACATCAAACAC-3' [SEQ ID NO:19];
3' *synC*: 5'-GCTACGACAGATGCAAAGGCG-3' [SEQ ID NO:20];
5' *synD*: 5'-AGAGGATTGGCTATTACATATAGC-3' [SEQ ID NO:21];
3' *synD*: 5'AGCTCTGTTGTCGATTACTCTCC-3' [SEQ ID NO:22];
5' *FKBP*: 5'-CATTACACAGGTTGGCTGGAAGACGG-3' [SEQ ID NO:23];
3' *FKBP*: 5'-GCAGCTCGACTTCAAATATCAAAGTGGC-3' [SEQ ID NO:24];
5' *recA*: 5'-GCCAGCAGGAAGAAAACCTCG-3' [SEQ ID NO:25];
3' *recA*: 5'-GCCGTTGTAGCTGTACCACGC-3' [SEQ ID NO:26];
5' *ctrA-synX*: 5'-CACCACCAAACAATACTGCC-3' [SEQ ID NO:27];
3' *ctrA-synX*: 5'-GCTTGTTCATTTGCTACCAAGTGG-3' [SEQ ID NO:28];
5' *galE*: 5'-CCAGCATCAATATCCTGCCACG-3' [SEQ ID NO:29];
3' *galE*: 5'-CCATCATTTGTGCAAGGCTGCG-3' [SEQ ID NO:30].

Nucleotide sequences were analyzed using either the DNASTAR (DNASTAR, Inc.) sequence analysis software or the Genetics Computer Group (GCG) Sequence Analysis Software Package, Version 7.3.1 UNIX [Devereux et al. (1984) *Nucl. Acids Res.* **12**:387-395]. Plate transformations of meningococcal strains were performed as described in Swartley et al. (1993) *Mol. Microbiol.* **10**:361-369.

For primer extension, the avian myeloblastosis virus reverse transcriptase (RT) primer extension system (Promega) was used according to the manufacturer's directions. Briefly, an antisense primer predicted to bind approximately 100 nucleotides from the 5' end of the mRNA transcript was 5' end labeled with [γ-^{32}P]ATP and polynucleotide kinase. The primer extension reaction mixture contained 100 fmol of the labeled primer, 40 µg of whole-cell RNA, and 1 U of avian myeloblastosis virus RT in an appropriate buffer. The labeled primer directed cDNA synthesis of the mRNA transcript with avian myeloblastosis virus RT. cDNA

synthesis continued to the 5' end of the RNA transcript, where it terminated, resulting in a labeled cDNA molecule of precisely defined length. The primer extension reaction mixtures, along with a standard dideoxy DNA sequencing reaction mixture catalyzed by the extension primer on control template DNA, were then run on an 8% polyacrylamide sequencing gel in order to define the precise nucleotide start site of the cDNA product. After electrophoresis, the gel was harvested and autoradiographed with X-ray film.

The following primers were used for primer extensions as described above. The 3' end of primer LJ6 (5'-CATCCTACAATTAAACTTCCACAC-3' [SEQ ID NO:31]) anneals 44 nucleotides downstream of the putative *ctrA* start codon (GTG) and was used to define the *ctrA* transcriptional start site. The 3' end of primer JS56 (5'-GAATACTAATTATACTCTAC GTACTC-3' [SEQ ID NO:32]) anneals 72 nucleotides upstream of the putative *synX* start codon (ATG) and was used to define the *synX* transcriptional start site.

Example 11: Nucleic Acid Purification

Chromosomal DNA was isolated as described by DiLella and Woo (1987) *Meth. Enzymol.* **152**:199-212. RNA from whole bacterial cells was prepared using a modification of the method of Baker et al. (1968) Proc. Natl. Acad. Sci. USA **60**:313-320, and Swartley et al. (1996) *J. Bacteriol.* **178**:4052-4059.

Example 12: Standard PCR and Single-Specific-Primer (SSP)-PCR

Standard PCR reactions were performed as described by Swartley et al. (1993) *Mol. Microbiol.* **10**:299-310. Oligonucleotide primers used are given in Table 1 and Example 10. Amplified products were visualized by 1.2% agarose gel electrophoresis and UV detection after ethidium bromide staining. PCR products were purified by passage through Qiaquick PCR-purification Spin Columns (Qiagen, Chatsworth, CA) prior to further manipulations. Chromosome walking via single-specific-primer (SSP)-PCR was performed as described by Shyamala and Ames (1989) *Gene* **84**:1-8).

Example 13: Primer Extension and Reverse Transcriptase (RT)-PCR

The AMV Reverse Transcriptase Primer Extension System (Promega) was used

according to the manufacturer's directions. A reverse transcriptase (RT)-PCR assay was carried out as previously described [Swartley et al. (1996) *supra*].

Example 14: Colony PCR

A single colony from a plated culture was collected using a sterile loop and resuspended in 20 µl of sterile distilled water. The colony suspension was then subjected to two rounds of freeze-thawing using a dry ice-ethanol bath and a 37°C water bath. One microliter of the freeze-thaw mixture was then used as template in standard PCR.

Example 15: Cloning of PCR Products

DNA products amplified using standard PCR or SSP-PCR were cloned using the TA Cloning® Kit (Invitrogen) or the pGEM®-T Vector System (Promega).

Example 16: Nucleotide Sequencing

Purified plasmid DNA and PCR products were sequenced by both manual and automated means. Oligonucleotide primers used are shown in Table 2. For manual sequencing the AmpliTaq Cycle Sequencing Kit (Perkin Elmer) was used according to the manufacturer's directions. Automated DNA sequencing was performed using the Prism Dye-Termination Cycle Sequencing Kit (Applied Biosystems) and completed reactions were run on an Applied Biosystems Model 377 Automated DNA Sequencer.

Example 17: Computer Sequence Analysis

Nucleotide and amino acid sequence analysis was performed using either the DNASTAR sequence analysis package (DNASTAR, Inc.) or the Genetics Computer Group (GCG) sequence analysis software package version 7.3.1-UNIX [Devereaux et al. (1984) *Nucl. Acids Res.* **12**:387-395].

Example 18: Polar and Nonpolar Insertional Mutagenesis

Polar mutagenesis of defined genes was conducted by insertion of an Ω-spectinomycin resistance cassette derived from *pHP45* [Prentki, P. and Krisch, H.M. (1984) *Gene* **29**:303-313]. Briefly, the genetic region to be interrupted was amplified by PCR from chromosomal

DNA and then cloned into *E. coli*. The plasmid containing the cloned PCR product was then linearized at a unique, blunt-ended restriction site present in the insert. A blunt *SmaI* fragment derived from *pHP45*, containing the entire Ω-spectinomycin resistance cassette, was then ligated into the cloned product and transformed into *E. coli* with selection for spectinomycin resistance. Putative transformants were checked by colony PCR to confirm assembly of appropriate constructs. Plasmid DNA was prepared from confirmed transformants and used to transform serogroup A strain F8229 with selection for spectinomycin resistance. Putative meningococcal transformants were checked by colony PCR and Southern DNA hybridization to confirm acquisition of the polar Ω-insertion mutation by homologous recombination. Primers JS102 and JS103 were used to amplify a 600 bp PCR fragment from the 5' end of the F8229 ORF1 which was subsequently cloned in *E. coli*. This product contained a unique *Stu*I restriction site located 356 bp downstream of the predicted ORF1 start codon. A *Sma*I fragment from pHP45, encoding the Ω-spectinomycin resistance cassette, was inserted into the unique *Stu*I site, and the resulting recombinant plasmid was used to transform wild-type serotype A strain F8229. Spectinomycin-resistant transformants were selected and acquisition of the Ω-insertion was confirmed by colony PCR and Southern hybridization.

The same approach was used to introduce Ω-spectinomycin resistance cassettes into ORF2, ORF3 and ORF4. To inactivate ORF2, a 451 bp DNA fragment derived from ORF2 was PCR amplified from strain F8229 using primers JS104 and JS105. An Ω-fragment was inserted into a unique *Hinc*II site present in the cloned PCR product (located 729 bp from the putative ORF2 start codon), and the resulting plasmid was transformed into strain F8229. Primers SE57 and SE61 were used to amplify an 858 bp product from ORF3, containing a unique *Ssp*i site located 507 bp downstream of the ORF3 start codon. Again, an Ω-fragment was inserted into this cloning site, and the construct was transformed into F8229. Finally, a 765 bp product was amplified from ORF4 using primers SE63 and SE56. The unique *Ssp*I cloning site in this product was located 159 bp from the putative ORF4 start codon. An Ω-fragment was inserted into the cloning site, and the construct was transformed into F8229.

Nonpolar mutants were created using the same allelic exchange technique described above; however, instead of using a polar Ω-fragment, a non-polar *aphA*-3 kanamycin resistance cassette derived from pUC18K [Menard, R. et al. (1993) *J. Bacteriol.* 175:5899-

5906] was inserted into the genetic region to be mutated. The orientation of the *aphA-3* insertion was checked by colony PCR and direct DNA sequencing to ensure that the cassette was fused in frame to the downstream sequences.

Example 19: DNA Transformation Procedures

Serogroup A meningococcal strain F8229 was transformed using the semi-quantitative transformation assay of Janik et al. (1976) *J. Clin. Microbiol.* 4:71-81. Chemical transformation of *E. coli* was performed as described by Chung et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:2172-2175.

Example 20: Southern DNA Hybridization

The Genius non-radioactive DNA labeling and detection system (Boerhinger Mannheim) was used. Specific DNA probes were PCR amplified, labeled with digoxigenin and used to probe Southern DNA blots according to the manufacturer's protocols.

Example 21: Capsule Quantitation by Colony Immunoblot and Whole Cell ELISA

Colony immunoblots were performed using the anti-serogroup A monoclonal antibody 14-1-A (generously provided by Dr. Wendell Zollinger, Walter Reed Army Institute of Research). Whole cell ELISA was performed using the method of Abdillahi and Poolman (1987) FEMS Microbiol. Lett. 48:367-371. Briefly, strains to be assayed were grown overnight on GC agar plates. Plate growth was then harvested and suspended in 5 ml of PBS containing 0.02% sodium azide. The cells were heat-inactivated at 56 for 30 minutes, then adjusted to an A_{650} of 0.1 and stored at 4°C until needed. To perform the ELISA, 100 µl of the cell suspension was added to a flat-bottomed microtiter plate (NUNC Maxi-sorp or Poly-sorp) and evaporated overnight at 33°C. The plate was then washed three times with a 0.05% solution of Tween 80 in sterile water. One hundred microliters of monoclonal antibody 14-1-A (diluted 1:10,000 in PBS containing 0.01% Tween 80 and 0.3% Casamino acids) was added to each well and the plate was incubated at 33°C for one hour. After a three-fold wash, 100 µl of goat anti-mouse IgA,G,M alkaline phosphatase conjugated antibody was added (diluted 1:10,000 in the above buffer) and incubated for 90 minutes at 33°C. The plate was washed three times, and 200 µl of substrate (1 mg p-nitrophenyl phosphate dissolved per ml

of 0.5M diethanolamine buffer containing 0.5 mM $MgCl_2$, pH 9.8) was added and left to stand at room temperature for 20-45 minutes. The reaction was stopped by the addition of 50 µl 3N NaOH and the A_{405} of each well was read using a BIO-TEK(BIO-TEK Instruments, Winoski, VT) model EL 312e automated plate reader.

```
          1                                                          60
F8229     MKVLTVFGTR PEAIKMAPVI LELQKHNTIT SKVCITAQHR EMLDQVLSLF EIKADYDLNI
F8239     MKVLTVFGTR PEAIKMAPVI LELQKHNTIT SKVCITAQHR EMLDQVLSLF EIKADYDLNI
NfrC      vKVLTVFGTR PEAIKMAPlV haLaKdpffe aKVCVTAQHR EMLDQVLkLF sIvpDYDLNI

          61                                                         120
          MKPNQSLQEI TTNIISSLTD VLEDFKPDCV LAHGDTTTTF AASLAAFYQK IPVGHIEAGL
          MKPNQSLQEI TTNIISSLTD VLEDFKPDCV LAHGDTTTTF AASLAAFYQK IPVGHIEAGL
          MqPgQgLtEI TcrIlegLkp ILaEFKPDvV LvHGDTTTTL AtSLAAFYQr IPVGHVEAGL

          121                                                        180
          RTYNLYSPWP EEANRRLTSV LSQWHFAPTE DSKNNLLSES IPSDKVIVTG NTVIDALM.V
          RTYNLYSPWP EEANRRLTSV LSQWHFAPTE DSKNNLLSES IPSDKVIVTG NTVIDALM.V
          RTgdLYSPWP EEANRtLTgh LamYHFsPTE tSrqNLLrEn VadsrIfITG NTVIDALLwV

          181                                                        240
          SLEKLKITTI KKQMEQAFPF IQDNSKVILI TAHRRENHGE GIKNIGLSIL ELAKKYPTFS
          SLEKLKITTI KKQMEQAFPF IQDNSKVILI TAHRRENHGE GIKNIGLSIL ELAKKYPTFS
          rdqvMssdkl rseLaanYPF IdpdkKmILV TgHRREsfGr GfeeIchala DIAtthqdIq

          241                                                        300
          FVIPLHLNPN VRkPIqdlLs sVhNVhLIEP QEYLPFVYLM skshIILsDS GGIQEEAPSL
          FVhsapFks. .......... .......... .......... .......... ..........
          IVyPvHLNPN VRePVnriLg hVkNVILIDP QEYLPFVWLM nhawlILtDS GGIQEEAPSL

          301                                                        360
          GKPVLVLRDT TERPEAVaAG TVkLVGsEtQ nIIEsfTqLI eypeyYekMa nIeNPYGIGn
          .......... .......... .......... .......... .......... ..........
          GKPVLVMRDT TERPEAVtAG TVrLVGtDkQ rIVEevTrLL kdeneYqaMs rahNPYGdGq

          361
          AskIIvEtLl kNR....
          .......... .......
          AcsrIlEaLk nNRIsl
```

Table 5. Amino Acid Sequence Comparison of the ORF1 Protein of Serogroup A *N. meningitidis* strain F8229, the truncated ORF1 gene product from unencapsulated *N. meningitidis* strain F8239 and the NfrC Protein from *Escherichia coli* (Pretty amino acid comparison program of the Genetics Computer Group sequence analysis software package, version 7.31). Identical or conserved amino acids are shown in upper case, and positions with amino acid differences are shown in lower case. Dots (..) Indicate gaps introduced by the software to facilitate the alignment.

```
                                                                                                                    110
1
mfilnnrkwr klkrdpsaff rdskfnflrY fsakkfaknF knsshihktn IskaqSniSs TlkEnrkqDM ll...piNff nfeYivkkln nqnalgvyIL psNltLkPAl
F8229 ORF2
cpsY
ORF5
..... ..... ...mskivsC eddrpvrrtL epiivtrqgk VarleS..Sl TphEaqieDL lfirkalNra dipFlflrnh knrp....VL aiNikLrPAv
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....

                                                                                                                    220
111
c...lleshk EdflnKflIT IssenLklqy kfnGQlknpk svneIWtdlf sIAhvdmkLs tdrtlsssiS qFwfrLeFck EDkdFIlfst aNrYsRKlWk hslknnqlfK
eralVtacas EpmyaK...T IdergLspvl vakGQlsqsl dprlVRlyrr rlApggfrFg .....S rFgveLqFWs fEetLIRcpv eNsLtRKvLP rkeVtpatlK
..... ..... ..... ..... ..... ..... .....m nLnklrkFFr EshlFfR... ..dFlnKrFP pynVeqplpe

                                                                                                                    330
221
egirnYseIs sL..PyeEDh ..NFDIDLVF TWVnseDKnW QE.....Lyk kykpDfnsdA TstsRFlsrD ELKFALRSwe MsgsFIRkIF IVsncaPPaW LDlNnPkIqw
lygYkWhtIE gHFtPhasDV ..tFDIDLVF sWVDgsDpeF ra...Rraae mshhvVGegd dadARlrqiD ELKYALRSVn MFaPWIRrIF IaTdslPPsW La.dhPmIti
teeFtlinaD anLasltEnV lpNFpIDvVF TWVDntDKaW QEqyyRtLqp ldqeDIGlyA TdpARFsnhn ELfYsvqaVq kFmPWVRnIF IVTadqkPkW LDeNlhskIk

                                                                                                                    440
331
VyhEEiHpq. SALPTFsSHA IEtsLHhIPg lSnyFIYSND DfLLtkPLnk dnFFysNGIA kLrlEAwgnV n.CectegeP dYlHgARnAn tLLekeFkkF .tTklhtHsp
VpaEDhFsDr SALPTYNSHA VEsqLHrIPD LSEHFlYSND DmFFgRPLka smFFspgGVt rF.IEAKtrI gLGtndptrs gFeNAARvnr qLLlRrFgqL .ITrhLeHTt
linhsqLlDa kyLPTFNSHv IEanLykIPD LSEHFIYfND DvFvaRPLmp nhFFenNGlA sLfVanKsfq kMrqrglitP tL.tAsehAl kLLkRhYpti nIntpLvHTy

                                                                                                                    550
441
qsMRtdILFE MEkkYPEEFN RTLhNKFRSl dDIaVTgYLY HhYALLsGRA lQssDkteLv qqnhdFkkkL NnvvtLtKER NFDkLPlSvC INDGadShl. neEWnvqVIk
VPLRKsVLFE MEqeFPEEFa RTqesvFRSg tDIsVTnsLY HYYALltGRA vQqekakvLY vdttSY.tgL NllpeLrKrR NYDfF....C lNDGSfpevp atERAerVVs
VPLRKtsFqk awslFeDEiN sflnNKvRhn sElnmasFLi pWlmyLdGyA tpkrEicyyF nirsShaqtq ykkllFeKEh lh..MPhSfC INDsS.Snna dknYAlhfrn

551                 574
FLETlFPlPs sFEK..... ....
FLErVFPIPa pWEKvatdfn rqdf
FMDTYFelet e..... ....
```

Table 6. Amino Acid Sequence Comparison of the ORF2 Protein of Serogroup A *N. meningitidis* strain F8229, the putative *epsY* gene product from *Mycobacterium leprae* and the putative amino acid sequence of ORF5 from Serogroup B from *N. meningitidis* strain B1903. (Pretty amino acid comparison program of the Genetics Computer Group sequence analysis software package, version 7.31). Identical or conserved amino acids are shown in upper case, and positions with amino acid differences are shown in lower case. Dots (..) indicate gaps introduced by the software to facilitate the alignment.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: EMORY UNIVERSITY

(ii) TITLE OF INVENTION: Serogroup-Specific Nucleotide Sequences in the Molecular Typing of Bacterial Isolates and the Preparation of Vaccines Thereto

(iii) NUMBER OF SEQUENCES: 51

(iv) CORRESPONDENCE ADDRESS:

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(B) STREET: 5370 Manhattan Circle, Suite 201

(C) CITY: Boulder

(D) STATE: Colorado

(E) COUNTRY: US

(F) ZIP: 80303

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US Unassigned

(B) FILING DATE: 23-SEP-1997

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/827,622

(B) FILING DATE: 09-APR-1997

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Ferber, Donna M.

(B) REGISTRATION NUMBER: 33,878

(C) REFERENCE/DOCKET NUMBER: 77-97 WO

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (303) 499-8080

(B) TELEFAX: (303) 499-8089

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 319 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTCCGGAGAT AACCTATGGG TTAAACGCCC AGGCAATGGA GACTTCAGCG TCAACGAATA 60

TGAAACATTA TTTGGTAAGG TCGCTGCTTG CAATATTCGC AAAGGTGCTC AAATCAAAAA 120

AACTGATATT GAATAATGCT TATTAACTTA GTTACTTTAT TAACAGAGGA TTGGCTATTA 180

CATATAGCTA ATTCTCATTA ATTTTTAAGA GATACAATAA TGCTAAAGAA AATAAAAAAA 240

GCTCTTTTTC AGCCTAAAAA GTTTTTTCAA GATTCAATGT GGTTGACAAC ATCTCCATTT 300

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 315 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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AACTGATATT GAATAAAAAT CTATAAATTG ACTCAATTTA ATGATAATCG GCTGACTTTT 180

CAGTCGATTA TCATTAAAAA TATACGGAAA AACAAATGTT GCAGAAAATA AGAAAAGCTC 240

TCTTCCACCC AAAAAAATTC TTCCAAGATT CCCAGTGGTT TGCAACACCT TTATTTAGCA 300

GCTTCGCACC CAAAA 315

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 319 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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AACTGATATT AGTTAATAAT AAAATAGATT AAGCTATTCT TAAATTCAGA ATATTGCTTA 180

TCTATATTAA AAATTTCTAA TTTTTAAGGT TCTGATTGAA ATCAGAACCT TATTTCAACT 240

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TATTTGTTAA CGGAATTCG 319

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 319 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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AACTGATATT AGTTAATAAT AAAATAGATT AAGCTATTCT TAAATTCAGA ATATTGCTTA 180

TCTATATTAA AAATTTCTAA TTTTTAAGGT TCTGATTGAA ATCAGAACCT TATTTCAACT 240

ATTACTTTTT ACTCATAATC GAATTATATA CTTTAGGACT TTATAATATG GCTGTTATTA 300

TATTTGTTAA CGGAATTCG 319

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 320 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Consensus sequence generated from sequence comparison of SEQ ID NOs:1-4."

(iii) HYPOTHETICAL: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 141..142
(D) OTHER INFORMATION: /note= "At nucleotide 141, N can be A, T, C or G or no nucleotide."

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 157..158
(D) OTHER INFORMATION: /note= "At nucleotide 157, N can be A, T, C or G or no nucleotide."

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 182..183
(D) OTHER INFORMATION: /note= "At nucleotides 182 and 183, N can be A, T C or G or no nucleotide."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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TGAAACATTA TTTGGTAAGG TCGCTGCTTG CAATATTCGC AAAGGTGCTC AAATCAAAAA 120

AACTGATATT NNNTAATAAT NNANTANNTT ANNCNANTTN TTAANNNNNG ANTNNNNNTT 180

ANNTATANTN AANNNTNNTN ANTTTTAANG NNNTNANNNA ANNCNGAANN NNATNNNAAN 240

NNNTNNTTTT NACNCANAAN NGNNTTNTNN ANNTTNNNAN TNNNTNANAN NNCNNTTATT 300

NTATNTNTTN NCNNNANNNN 320

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 279 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTCCGGAGAT AACCTATGGG TTAAACGCCC AGGCAATGGA GACTTCAGCG TCAACGAATA 60

TGAAACATTA TTTGGTAAGG TCGCTGCTTG CAATATTCGC AAAGGTGCTC AAATCAAAAA 120

AACTGATATT GAATAATGCT TATTAACTTA GTTACTTTAT TAACAGAGGA TTGGCTATTA 180

CATATAGCTA ATTCTCATTA ATTTTTAAGA GATACAATAA TGCTAAAGAA AATAAAAAAA 240

GCTCTTTTTC AGCCTAAAAA GTTTTTTCAA GATTCAATG 279

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 275 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GTCCGGAGAT AACCTATGGG TTAAACGCCC AGGCAATGGA GACTTCAGCG TCAACGAATA 60

TGAAACATTA TTTGGTAAAA TTGCTGCTTG TGATATTCGC AAAGGTGCTC AAATCAAAAA 120

AACTGATATC GAATAAAAAT CTATAAATTG ACTCAATTTA ATGATAATCG GCTGACTTTT 180

CAGTCGATTA TCATTAAAAA TATACGGAAA AACAAATGTT GCAGAAAATA AGAAAAGCTC 240

TCTTCCACCC AAAAAAATTC TTCCAAGATT CCCAG 275

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5064 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 479..1597

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1599..3236

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3309..4052

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 4054..4917

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AATACATCAC CAATATTTAG CGTACCGGTA GAAGCATAAC CATCGCCAAA CTGGGTAAAA 60

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TCTACTTCAG GAATTTGAGC TTCAGACTGT TGCCCCAATG AGACAACTTT TTTTGCACTT 180

GGGCCAGAGG AGGGAATAGC ACTACATGCA CTTCCCAAAA TTAAAAAAGA AATTACAATA 240

CAAAACTTTA ACTTAAGCAT AAAATAAAAA ATCTCATTAA GTATGATTGT TTTTAAATAA 300

ATTTAAAACC TACCAGAGAT ACAATACCAC TTTATTTTGT AGAACACAAA CGTGTATAAT 360

ATATGACATA AACATCATCT TCGAAATAAT ATTGGGGCTT AGGAAGCAAA ATCATCAAAA 420

AACGTGATAA GCTCCTAATA TTTTTAACAC ATTACTATAT TACACATAGG ATATTCCA 478

ATG AAA GTC TTA ACC GTC TTT GGC ACT CGC CCT GAA GCT ATT AAA ATG 526
Met Lys Val Leu Thr Val Phe Gly Thr Arg Pro Glu Ala Ile Lys Met
1 5 10 15

GCG CCT GTA ATT CTA GAG TTA CAA AAA CAT AAC ACA ATT ACT TCA AAA 574
Ala Pro Val Ile Leu Glu Leu Gln Lys His Asn Thr Ile Thr Ser Lys
20 25 30

```
GTT TGC ATT ACT GCA CAG CAT CGT GAA ATG CTA GAT CAG GTT TTG AGC      622
Val Cys Ile Thr Ala Gln His Arg Glu Met Leu Asp Gln Val Leu Ser
      35                  40                  45

CTA TTC GAA ATC AAA GCT GAT TAT GAT TTA AAT ATC ATG AAA CCC AAC      670
Leu Phe Glu Ile Lys Ala Asp Tyr Asp Leu Asn Ile Met Lys Pro Asn
     50                  55                  60

CAG AGC CTA CAA GAA ATC ACA ACA AAT ATC ATC TCA AGC CTT ACC GAT      718
Gln Ser Leu Gln Glu Ile Thr Thr Asn Ile Ile Ser Ser Leu Thr Asp
 65                  70                  75                  80

GTT CTT GAA GAT TTC AAA CCT GAC TGC GTC CTT GCT CAC GGA GAC ACC      766
Val Leu Glu Asp Phe Lys Pro Asp Cys Val Leu Ala His Gly Asp Thr
                 85                  90                  95

ACA ACA ACT TTT GCA GCT AGC CTT GCT GCA TTC TAT CAA AAA ATA CCT      814
Thr Thr Thr Phe Ala Ala Ser Leu Ala Ala Phe Tyr Gln Lys Ile Pro
            100                 105                 110

GTT GGC CAC ATT GAA GCA GGC CTG AGA ACT TAT AAT TTA TAC TCT CCT      862
Val Gly His Ile Glu Ala Gly Leu Arg Thr Tyr Asn Leu Tyr Ser Pro
        115                 120                 125

TGG CCA GAG GAA GCA AAT AGG CGT TTA ACA AGC GTT CTA AGC CAG TGG      910
Trp Pro Glu Glu Ala Asn Arg Arg Leu Thr Ser Val Leu Ser Gln Trp
    130                 135                 140

CAT TTT GCA CCT ACT GAA GAT TCT AAA AAT AAC TTA CTA TCT GAA TCA      958
His Phe Ala Pro Thr Glu Asp Ser Lys Asn Asn Leu Leu Ser Glu Ser
145                 150                 155                 160

ATA CCT TCT GAC AAA GTT ATT GTT ACT GGA AAT ACT GTC ATA GAT GCA     1006
Ile Pro Ser Asp Lys Val Ile Val Thr Gly Asn Thr Val Ile Asp Ala
                165                 170                 175
```

CTA ATG GTA TCT CTA GAA AAA CTA AAA ATA ACT ACA ATT AAA AAA CAA 1054
Leu Met Val Ser Leu Glu Lys Leu Lys Ile Thr Thr Ile Lys Lys Gln
180 185 190

ATG GAA CAA GCT TTT CCA TTT ATT CAG GAC AAC TCT AAA GTA ATT TTA 1102
Met Glu Gln Ala Phe Pro Phe Ile Gln Asp Asn Ser Lys Val Ile Leu
195 200 205

ATT ACC GCT CAT AGA AGA GAA AAT CAT GGG GAA GGT ATT AAA AAT ATT 1150
Ile Thr Ala His Arg Arg Glu Asn His Gly Glu Gly Ile Lys Asn Ile
210 215 220

GGA CTT TCT ATC TTA GAA TTA GCT AAA AAA TAC CCA ACA TTC TCT TTT 1198
Gly Leu Ser Ile Leu Glu Leu Ala Lys Lys Tyr Pro Thr Phe Ser Phe
225 230 235 240

GTG ATT CCG CTC CAT TTA AAT CCT AAC GTT AGA AAA CCA ATT CAA GAT 1246
Val Ile Pro Leu His Leu Asn Pro Asn Val Arg Lys Pro Ile Gln Asp
245 250 255

TTA TTA TCC TCT GTG CAC AAT GTT CAT CTT ATT GAG CCA CAA GAA TAC 1294
Leu Leu Ser Ser Val His Asn Val His Leu Ile Glu Pro Gln Glu Tyr
260 265 270

TTA CCA TTC GTA TAT TTA ATG TCT AAA AGC CAT ATA ATA TTA AGT GAT 1342
Leu Pro Phe Val Tyr Leu Met Ser Lys Ser His Ile Ile Leu Ser Asp
275 280 285

TCA GGC GGC ATA CAA GAA GAA GCT CCA TCC CTA GGA AAA CCA GTT CTT 1390
Ser Gly Gly Ile Gln Glu Glu Ala Pro Ser Leu Gly Lys Pro Val Leu
290 295 300

GTA TTA AGA GAT ACT ACA GAA CGT CCT GAA GCT GTA GCT GCA GGA ACT 1438
Val Leu Arg Asp Thr Thr Glu Arg Pro Glu Ala Val Ala Ala Gly Thr
305 310 315 320

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GTA AAA TTA GTA GGT TCT GAA ACT CAA AAT ATT ATT GAG AGC TTT ACA      1486
Val Lys Leu Val Gly Ser Glu Thr Gln Asn Ile Ile Glu Ser Phe Thr
                325                 330                 335

CAA CTA ATT GAA TAC CCT GAA TAT TAT GAA AAA ATG GCT AAT ATT GAA      1534
Gln Leu Ile Glu Tyr Pro Glu Tyr Tyr Glu Lys Met Ala Asn Ile Glu
            340                 345                 350

AAC CCT TAC GGG ATA GGT AAT GCC TCA AAA ATC ATT GTA GAA ACT TTA      1582
Asn Pro Tyr Gly Ile Gly Asn Ala Ser Lys Ile Ile Val Glu Thr Leu
        355                 360                 365

TTA AAG AAT AGA TAA A ATG TTT ATA CTT AAT AAC AGA AAA TGG CGT        1628
Leu Lys Asn Arg  *    Met Phe Ile Leu Asn Asn Arg Lys Trp Arg
    370                 1               5                  10

AAA CTT AAA AGA GAC CCT AGC GCT TTC TTT CGA GAT AGT AAA TTT AAC      1676
Lys Leu Lys Arg Asp Pro Ser Ala Phe Phe Arg Asp Ser Lys Phe Asn
                15                  20                  25

TTT TTA AGA TAT TTT TCT GCT AAA AAA TTT GCA AAG AAT TTT AAA AAT      1724
Phe Leu Arg Tyr Phe Ser Ala Lys Lys Phe Ala Lys Asn Phe Lys Asn
            30                  35                  40

TCA TCA CAT ATC CAT AAA ACT AAT ATA AGT AAA GCT CAA TCA AAT ATT      1772
Ser Ser His Ile His Lys Thr Asn Ile Ser Lys Ala Gln Ser Asn Ile
        45                  50                  55

TCT TCA ACC TTA AAA GAA AAT CGG AAA CAA GAT ATG TTA ATT CCT ATT      1820
Ser Ser Thr Leu Lys Glu Asn Arg Lys Gln Asp Met Leu Ile Pro Ile
    60                  65                  70

AAT TTT TTT AAT TTT GAA TAT ATA GTT AAA AAA CTT AAC AAT CAA AAC      1868
Asn Phe Phe Asn Phe Glu Tyr Ile Val Lys Lys Leu Asn Asn Gln Asn
75                  80                  85                  90
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GCA ATA GGT GTA TAT ATT CTT CCT TCT AAT CTT ACT CTT AAG CCT GCA 1916
Ala Ile Gly Val Tyr Ile Leu Pro Ser Asn Leu Thr Leu Lys Pro Ala
95 100 105

TTA TGT ATT CTA GAA TCA CAT AAA GAA GAC TTT TTA AAT AAA TTT CTT 1964
Leu Cys Ile Leu Glu Ser His Lys Glu Asp Phe Leu Asn Lys Phe Leu
110 115 120

CTT ACT ATT TCC TCT GAA AAT TTA AAG CTT CAA TAC AAA TTT AAT GGA 2012
Leu Thr Ile Ser Ser Glu Asn Leu Lys Leu Gln Tyr Lys Phe Asn Gly
125 130 135

CAA ATA AAA AAT CCT AAG TCC GTA AAT GAA ATT TGG ACA GAT TTA TTT 2060
Gln Ile Lys Asn Pro Lys Ser Val Asn Glu Ile Trp Thr Asp Leu Phe
140 145 150

AGC ATT GCT CAT GTT GAC ATG AAA CTC AGC ACA GAT AGA ACT TTA AGT 2108
Ser Ile Ala His Val Asp Met Lys Leu Ser Thr Asp Arg Thr Leu Ser
155 160 165 170

TCA TCT ATA TCT CAA TTT TGG TTC AGA TTA GAG TTC TGT AAA GAA GAT 2156
Ser Ser Ile Ser Gln Phe Trp Phe Arg Leu Glu Phe Cys Lys Glu Asp
175 180 185

AAG GAT TTT ATC TTA TTT TCT ACA GCT AAC AGA TAT TCT AGA AAA CTT 2204
Lys Asp Phe Ile Leu Phe Ser Thr Ala Asn Arg Tyr Ser Arg Lys Leu
190 195 200

TGG AAG CAC TCT ATT AAA AAT AAT CAA TTA TTT AAA GAA GGC ATA CGA 2252
Trp Lys His Ser Ile Lys Asn Asn Gln Leu Phe Lys Glu Gly Ile Arg
205 210 215

AAC TAT TCA GAA ATA TCT TCA TTA CCC TAT GAA GAA GAT CAT AAT TTT 2300
Asn Tyr Ser Glu Ile Ser Ser Leu Pro Tyr Glu Glu Asp His Asn Phe
220 225 230

GAT ATT GAT TTA GTA TTT ACT TGG GTC AAC TCA GAA GAT AAG AAT TGG 2348
Asp Ile Asp Leu Val Phe Thr Trp Val Asn Ser Glu Asp Lys Asn Trp
235 240 245 250

CAA GAG TTA TAT AAA AAA TAT AAG CCC GAC TTT AAT AGC GAT GCA ACC 2396
Gln Glu Leu Tyr Lys Lys Tyr Lys Pro Asp Phe Asn Ser Asp Ala Thr
255 260 265

AGT ACA TCA AGA TTC CTT AGT AGA GAT GAA TTA AAA TTC GCA TTA CGC 2444
Ser Thr Ser Arg Phe Leu Ser Arg Asp Glu Leu Lys Phe Ala Leu Arg
270 275 280

TCT TGG GAA ATG AGT GGA TCC TTC ATT CGA AAA ATT TTT ATT GTC TCT 2492
Ser Trp Glu Met Ser Gly Ser Phe Ile Arg Lys Ile Phe Ile Val Ser
285 290 295

AAT TGT GCT CCC CCA GCA TGG CTA GAT TTA AAT AAC CCT AAA ATT CAA 2540
Asn Cys Ala Pro Pro Ala Trp Leu Asp Leu Asn Asn Pro Lys Ile Gln
300 305 310

TGG GTA TAT CAC GAA GAA ATT ATG CCA CAA AGT GCC CTT CCT ACT TTT 2588
Trp Val Tyr His Glu Glu Ile Met Pro Gln Ser Ala Leu Pro Thr Phe
315 320 325 330

AGC TCA CAT GCT ATT GAA ACC AGC TTG CAC CAT ATA CCA GGA ATT AGT 2636
Ser Ser His Ala Ile Glu Thr Ser Leu His His Ile Pro Gly Ile Ser
335 340 345

AAC TAT TTT ATT TAC AGC AAT GAC GAC TTC CTA TTA ACT AAA CCA TTG 2684
Asn Tyr Phe Ile Tyr Ser Asn Asp Asp Phe Leu Leu Thr Lys Pro Leu
350 355 360

AAT AAA GAC AAT TTC TTC TAT TCG AAT GGT ATT GCA AAG TTA AGA TTA 2732
Asn Lys Asp Asn Phe Phe Tyr Ser Asn Gly Ile Ala Lys Leu Arg Leu
365 370 375

GAA GCA TGG GGA AAT GTT AAT GGT GAA TGT ACT GAA GGA GAA CCT GAC 2780
Glu Ala Trp Gly Asn Val Asn Gly Glu Cys Thr Glu Gly Glu Pro Asp
380 385 390

TAC TTA AAT GGT GCT CGC AAT GCG AAC ACT CTC TTA GAA AAG GAA TTT 2828
Tyr Leu Asn Gly Ala Arg Asn Ala Asn Thr Leu Leu Glu Lys Glu Phe
395 400 405 410

AAA AAA TTT ACT ACT AAA CTA CAT ACT CAC TCC CCT CAA TCC ATG AGA 2876
Lys Lys Phe Thr Thr Lys Leu His Thr His Ser Pro Gln Ser Met Arg
415 420 425

ACT GAT ATT TTA TTT GAG ATG GAA AAA AAA TAT CCA GAA GAG TTT AAT 2924
Thr Asp Ile Leu Phe Glu Met Glu Lys Lys Tyr Pro Glu Glu Phe Asn
430 435 440

AGA ACA CTA CAT AAT AAA TTC CGA TCT TTA GAT GAT ATT GCA GTA ACG 2972
Arg Thr Leu His Asn Lys Phe Arg Ser Leu Asp Asp Ile Ala Val Thr
445 450 455

GGC TAT CTC TAT CAT CAT TAT GCC CTA CTC TCT GGA CGA GCA CTA CAA 3020
Gly Tyr Leu Tyr His His Tyr Ala Leu Leu Ser Gly Arg Ala Leu Gln
460 465 470

AGT TCT GAC AAG ACG GAA CTT GTA CAG CAA AAT CAT GAT TTC AAA AAG 3068
Ser Ser Asp Lys Thr Glu Leu Val Gln Gln Asn His Asp Phe Lys Lys
475 480 485 490

AAA CTA AAT AAT GTA GTG ACC TTA ACT AAA GAA AGG AAT TTT GAC AAA 3116
Lys Leu Asn Asn Val Val Thr Leu Thr Lys Glu Arg Asn Phe Asp Lys
495 500 505

CTT CCT TTG AGC GTA TGT ATC AAC GAT GGT GCT GAT AGT CAC TTG AAT 3164
Leu Pro Leu Ser Val Cys Ile Asn Asp Gly Ala Asp Ser His Leu Asn
510 515 520

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GAA GAA TGG AAT GTT CAA GTT ATT AAG TTC TTA GAA ACT CTT TTC CCA      3212
Glu Glu Trp Asn Val Gln Val Ile Lys Phe Leu Glu Thr Leu Phe Pro
      525                 530                 535

TTA CCA TCA TCA TTT GAG AAA TAA GTTAAATTAT GAAGAACCTT TGAGTGCAAT    3266
Leu Pro Ser Ser Phe Glu Lys  *
    540                 545

TCGAAGGTTC TTCATTCATA TTATTCATAT TTTGGAGAAA TT ATG TTA TCT AAT      3320
                                               Met Leu Ser Asn
                                                 1

TTA AAA ACA GGA AAT AAT ATC TTA GGA TTA CCT GAA TTT GAG TTG AAT      3368
Leu Lys Thr Gly Asn Asn Ile Leu Gly Leu Pro Glu Phe Glu Leu Asn
  5                  10                  15                  20

GGC TGC CGA TTC TTA TAT AAA AAA GGT ATA GAA AAA ACA ATT ATT ACT      3416
Gly Cys Arg Phe Leu Tyr Lys Lys Gly Ile Glu Lys Thr Ile Ile Thr
                  25                  30                  35

TTT TCA GCA TTT CCT CCT AAA GAT ATT GCT CAA AAA TAT AAT TAT ATA      3464
Phe Ser Ala Phe Pro Pro Lys Asp Ile Ala Gln Lys Tyr Asn Tyr Ile
             40                  45                  50

AAA GAT TTT TTA AGT TCT AAT TAT ACT TTT TTA GCA TTC TTA GAT ACC      3512
Lys Asp Phe Leu Ser Ser Asn Tyr Thr Phe Leu Ala Phe Leu Asp Thr
        55                  60                  65

AAA TAT CCA GAA GAT GAT GCT AGA GGC ACT TAT TAC ATT ACT AAT GAG      3560
Lys Tyr Pro Glu Asp Asp Ala Arg Gly Thr Tyr Tyr Ile Thr Asn Glu
    70                  75                  80

TTA GAT AAT GGA TAT TTA CAA ACC ATA CAT TGT ATT ATT CAA TTA TTA      3608
Leu Asp Asn Gly Tyr Leu Gln Thr Ile His Cys Ile Ile Gln Leu Leu
 85                  90                  95                  100
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TCG AAT ACA AAT CAA GAA GAT ACC TAC CTT TTG GGT TCA AGT AAA GGT 3656
Ser Asn Thr Asn Gln Glu Asp Thr Tyr Leu Leu Gly Ser Ser Lys Gly
105 110 115

GGC GTT GGC GCA CTT CTA CTC GGT CTT ACA TAT AAT TAT CCT AAT ATA 3704
Gly Val Gly Ala Leu Leu Leu Gly Leu Thr Tyr Asn Tyr Pro Asn Ile
120 125 130

ATT ATT AAT GCT CCT CAA GCC AAA TTA GCA GAT TAT ATC AAA ACA CGC 3752
Ile Ile Asn Ala Pro Gln Ala Lys Leu Ala Asp Tyr Ile Lys Thr Arg
135 140 145

TCG AAA ACC ATT CTT TCA TAT ATG CTT GGA ACC TCT AAA AGA TTT CAA 3800
Ser Lys Thr Ile Leu Ser Tyr Met Leu Gly Thr Ser Lys Arg Phe Gln
150 155 160

GAT ATT AAT TAC GAT TAT ATC AAT GAC TTC TTA CTA TCT AAA ATT AAG 3848
Asp Ile Asn Tyr Asp Tyr Ile Asn Asp Phe Leu Leu Ser Lys Ile Lys
165 170 175 180

ACT TGC GAC TCC TCA CTT AAA TGG AAT ATT CAT ATA ACT TGC GGA AAA 3896
Thr Cys Asp Ser Ser Leu Lys Trp Asn Ile His Ile Thr Cys Gly Lys
185 190 195

GAT GAT TCA TAT CAT TTA AAT GAA TTA GAA ATT CTA AAA AAT GAA TTT 3944
Asp Asp Ser Tyr His Leu Asn Glu Leu Glu Ile Leu Lys Asn Glu Phe
200 205 210

AAT ATA AAA GCT ATT ACG ATT AAA ACC AAA CTA ATT TCT GGC GGG CAT 3992
Asn Ile Lys Ala Ile Thr Ile Lys Thr Lys Leu Ile Ser Gly Gly His
215 220 225

GAT AAT GAA GCA ATT GCC CAC TAT AGA GAA TAC TTT AAA ACC ATA ATC 4040
Asp Asn Glu Ala Ile Ala His Tyr Arg Glu Tyr Phe Lys Thr Ile Ile
230 235 240

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CAA AAT ATA TAA A ATG CGT AAG ATT ACT TTT ATT ATC CCT ATA AAA      4086
Gln Asn Ile  *    Met Arg Lys Ile Thr Phe Ile Ile Pro Ile Lys
245               1               5                  10

CAG TCT TTA ATA AAA CCT GAT TGC TTT ATA CGC CTC TTT TTT AAT TTA    4134
Gln Ser Leu Ile Lys Pro Asp Cys Phe Ile Arg Leu Phe Phe Asn Leu
            15                  20                  25

TTT TTG CTA AAA AAA TTC TCA AGT AAA TAC GGA TTT TCT ATA TTA GTT    4182
Phe Leu Leu Lys Lys Phe Ser Ser Lys Tyr Gly Phe Ser Ile Leu Val
        30                  35                  40

GCA GAC AAC AGT AAC TTC CTT TGG AAA AAT ATT ATT AAA TTA ATT ACA    4230
Ala Asp Asn Ser Asn Phe Leu Trp Lys Asn Ile Ile Lys Leu Ile Thr
    45                  50                  55

AAA TTT TAC AAA TGT AAT TAT ATT AGT ATT AAA TCT CAT AAT ACT TTT    4278
Lys Phe Tyr Lys Cys Asn Tyr Ile Ser Ile Lys Ser His Asn Thr Phe
60                  65                  70                  75

TAT ACG CCT GCT AAA ATT AAA AAT GCA GCT GCC ATC TAT AGT TTT AAT    4326
Tyr Thr Pro Ala Lys Ile Lys Asn Ala Ala Ala Ile Tyr Ser Phe Asn
                80                  85                  90

ACC TTG AAT TCA AAT TAC ATT TTA TTC TTA GAT GTT GAC GTT TTA TTA    4374
Thr Leu Asn Ser Asn Tyr Ile Leu Phe Leu Asp Val Asp Val Leu Leu
            95                  100                 105

TCG GAA AAT TTT ATC CAA CAT TTA ATA AAA AAA ACA AAA ACC AAT ATC    4422
Ser Glu Asn Phe Ile Gln His Leu Ile Lys Lys Thr Lys Thr Asn Ile
        110                 115                 120

GCC TTT GAT TGG TAC CCT GTT TCA TTC TTA AAC AAA CAA TTT GGG ATT    4470
Ala Phe Asp Trp Tyr Pro Val Ser Phe Leu Asn Lys Gln Phe Gly Ile
    125                 130                 135
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ATA AAT TTT ATA TTA TTC TCA TAT AAA GGT AAT CTA AAT ATA GAA GAA 4518
Ile Asn Phe Ile Leu Phe Ser Tyr Lys Gly Asn Leu Asn Ile Glu Glu
140 145 150 155

TCA TTC ATT ATA CAA ACA GGG TTT GTA ACT GGC TTA CAA TTA TTT AAT 4566
Ser Phe Ile Ile Gln Thr Gly Phe Val Thr Gly Leu Gln Leu Phe Asn
160 165 170

TCT GAT TTT TTC TAC AAA ACA GCT GGA TAC AAT GAA AGC TTT CTT GGC 4614
Ser Asp Phe Phe Tyr Lys Thr Ala Gly Tyr Asn Glu Ser Phe Leu Gly
175 180 185

TAT GGC TGT GAA GAT ATT GAA ATG ATT CAC AGA GCA ACA TTA TTA TTA 4662
Tyr Gly Cys Glu Asp Ile Glu Met Ile His Arg Ala Thr Leu Leu Leu
190 195 200

AAT ATT AGA CCT GCC TTT AAT GAA AAT CAT CAA TAT TTT ACA GAT GAT 4710
Asn Ile Arg Pro Ala Phe Asn Glu Asn His Gln Tyr Phe Thr Asp Asp
205 210 215

AGA GGA TAT ATG CCT TCT AAA TTA ACC GGA TTT CGA AAT TAT TTT TAT 4758
Arg Gly Tyr Met Pro Ser Lys Leu Thr Gly Phe Arg Asn Tyr Phe Tyr
220 225 230 235

TAT TTG AAA AGA GAT GAA TTT TCA AAC TTA CAG ATA ACT CCT AAA CAT 4806
Tyr Leu Lys Arg Asp Glu Phe Ser Asn Leu Gln Ile Thr Pro Lys His
240 245 250

TTC TGG CAT AAG CGA AAA AAT AAA TCA AAA TAT CTA AAA AAT AGA TAT 4854
Phe Trp His Lys Arg Lys Asn Lys Ser Lys Tyr Leu Lys Asn Arg Tyr
255 260 265

CAA AAT GAT GTA AAA ATG ATT CAG ATT ATG AAA GAT TTT GAT CGA AAA 4902
Gln Asn Asp Val Lys Met Ile Gln Ile Met Lys Asp Phe Asp Arg Lys
270 275 280

TTT CTA AAA AAT TAA CGAGCTGTCT TGCCCATATG AATCCTGATT ACTTTAATTT 4957
Phe Leu Lys Asn *
285

AATTATGAAA AATATTCTCG TTACCGGCGG CACCGGTTTT ATCGGCTCGC ACACCGTTGT 5017

TTCTTTGCTG AAAAGCGGCC ATCAAGTCGT GATTTTGGAT AACCTAT 5064

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 373 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Lys Val Leu Thr Val Phe Gly Thr Arg Pro Glu Ala Ile Lys Met
1 5 10 15

Ala Pro Val Ile Leu Glu Leu Gln Lys His Asn Thr Ile Thr Ser Lys
20 25 30

Val Cys Ile Thr Ala Gln His Arg Glu Met Leu Asp Gln Val Leu Ser
35 40 45

Leu Phe Glu Ile Lys Ala Asp Tyr Asp Leu Asn Ile Met Lys Pro Asn
50 55 60

Gln Ser Leu Gln Glu Ile Thr Thr Asn Ile Ile Ser Ser Leu Thr Asp
65 70 75 80

Val Leu Glu Asp Phe Lys Pro Asp Cys Val Leu Ala His Gly Asp Thr
85 90 95

Thr Thr Thr Phe Ala Ala Ser Leu Ala Ala Phe Tyr Gln Lys Ile Pro
100 105 110

Val Gly His Ile Glu Ala Gly Leu Arg Thr Tyr Asn Leu Tyr Ser Pro
115 120 125

Trp Pro Glu Glu Ala Asn Arg Arg Leu Thr Ser Val Leu Ser Gln Trp
130 135 140

His Phe Ala Pro Thr Glu Asp Ser Lys Asn Asn Leu Leu Ser Glu Ser
145 150 155 160

Ile Pro Ser Asp Lys Val Ile Val Thr Gly Asn Thr Val Ile Asp Ala
165 170 175

Leu Met Val Ser Leu Glu Lys Leu Lys Ile Thr Thr Ile Lys Lys Gln
180 185 190

Met Glu Gln Ala Phe Pro Phe Ile Gln Asp Asn Ser Lys Val Ile Leu
195 200 205

Ile Thr Ala His Arg Arg Glu Asn His Gly Glu Gly Ile Lys Asn Ile
210 215 220

Gly Leu Ser Ile Leu Glu Leu Ala Lys Lys Tyr Pro Thr Phe Ser Phe
225 230 235 240

Val Ile Pro Leu His Leu Asn Pro Asn Val Arg Lys Pro Ile Gln Asp
245 250 255

Leu Leu Ser Ser Val His Asn Val His Leu Ile Glu Pro Gln Glu Tyr
260 265 270

Leu Pro Phe Val Tyr Leu Met Ser Lys Ser His Ile Ile Leu Ser Asp
275 280 285

Ser Gly Gly Ile Gln Glu Glu Ala Pro Ser Leu Gly Lys Pro Val Leu
290 295 300

Val Leu Arg Asp Thr Thr Glu Arg Pro Glu Ala Val Ala Ala Gly Thr
305 310 315 320

Val Lys Leu Val Gly Ser Glu Thr Gln Asn Ile Ile Glu Ser Phe Thr
325 330 335

Gln Leu Ile Glu Tyr Pro Glu Tyr Tyr Glu Lys Met Ala Asn Ile Glu
340 345 350

Asn Pro Tyr Gly Ile Gly Asn Ala Ser Lys Ile Ile Val Glu Thr Leu
355 360 365

Leu Lys Asn Arg *
370

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 546 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Phe Ile Leu Asn Asn Arg Lys Trp Arg Lys Leu Lys Arg Asp Pro
1 5 10 15

Ser Ala Phe Phe Arg Asp Ser Lys Phe Asn Phe Leu Arg Tyr Phe Ser
20 25 30

Ala Lys Lys Phe Ala Lys Asn Phe Lys Asn Ser Ser His Ile His Lys
35 40 45

Thr Asn Ile Ser Lys Ala Gln Ser Asn Ile Ser Ser Thr Leu Lys Glu
50 55 60

Asn Arg Lys Gln Asp Met Leu Ile Pro Ile Asn Phe Phe Asn Phe Glu
65 70 75 80

Tyr Ile Val Lys Lys Leu Asn Asn Gln Asn Ala Ile Gly Val Tyr Ile
85 90 95

Leu Pro Ser Asn Leu Thr Leu Lys Pro Ala Leu Cys Ile Leu Glu Ser
100 105 110

His Lys Glu Asp Phe Leu Asn Lys Phe Leu Leu Thr Ile Ser Ser Glu
115 120 125

Asn Leu Lys Leu Gln Tyr Lys Phe Asn Gly Gln Ile Lys Asn Pro Lys
130 135 140

Ser Val Asn Glu Ile Trp Thr Asp Leu Phe Ser Ile Ala His Val Asp
145 150 155 160

Met Lys Leu Ser Thr Asp Arg Thr Leu Ser Ser Ser Ile Ser Gln Phe
165 170 175

Trp Phe Arg Leu Glu Phe Cys Lys Glu Asp Lys Asp Phe Ile Leu Phe
180 185 190

Ser Thr Ala Asn Arg Tyr Ser Arg Lys Leu Trp Lys His Ser Ile Lys
195 200 205

Asn Asn Gln Leu Phe Lys Glu Gly Ile Arg Asn Tyr Ser Glu Ile Ser
210 215 220

Ser Leu Pro Tyr Glu Glu Asp His Asn Phe Asp Ile Asp Leu Val Phe
225 230 235 240

Thr Trp Val Asn Ser Glu Asp Lys Asn Trp Gln Glu Leu Tyr Lys Lys
245 250 255

Tyr Lys Pro Asp Phe Asn Ser Asp Ala Thr Ser Thr Ser Arg Phe Leu
260 265 270

Ser Arg Asp Glu Leu Lys Phe Ala Leu Arg Ser Trp Glu Met Ser Gly
275 280 285

Ser Phe Ile Arg Lys Ile Phe Ile Val Ser Asn Cys Ala Pro Pro Ala
290 295 300

Trp Leu Asp Leu Asn Asn Pro Lys Ile Gln Trp Val Tyr His Glu Glu
305 310 315 320

Ile Met Pro Gln Ser Ala Leu Pro Thr Phe Ser Ser His Ala Ile Glu
325 330 335

Thr Ser Leu His His Ile Pro Gly Ile Ser Asn Tyr Phe Ile Tyr Ser
340 345 350

Asn Asp Asp Phe Leu Leu Thr Lys Pro Leu Asn Lys Asp Asn Phe Phe
355 360 365

Tyr Ser Asn Gly Ile Ala Lys Leu Arg Leu Glu Ala Trp Gly Asn Val
370 375 380

Asn Gly Glu Cys Thr Glu Gly Glu Pro Asp Tyr Leu Asn Gly Ala Arg
385 390 395 400

Asn Ala Asn Thr Leu Leu Glu Lys Glu Phe Lys Lys Phe Thr Thr Lys
405 410 415

Leu His Thr His Ser Pro Gln Ser Met Arg Thr Asp Ile Leu Phe Glu
420 425 430

Met Glu Lys Lys Tyr Pro Glu Glu Phe Asn Arg Thr Leu His Asn Lys
435 440 445

Phe Arg Ser Leu Asp Asp Ile Ala Val Thr Gly Tyr Leu Tyr His His
450 455 460

Tyr Ala Leu Leu Ser Gly Arg Ala Leu Gln Ser Ser Asp Lys Thr Glu
465 470 475 480

Leu Val Gln Gln Asn His Asp Phe Lys Lys Lys Leu Asn Asn Val Val
485 490 495

Thr Leu Thr Lys Glu Arg Asn Phe Asp Lys Leu Pro Leu Ser Val Cys
500 505 510

Ile Asn Asp Gly Ala Asp Ser His Leu Asn Glu Glu Trp Asn Val Gln
515 520 525

Val Ile Lys Phe Leu Glu Thr Leu Phe Pro Leu Pro Ser Ser Phe Glu
530 535 540

Lys *
545

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 248 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Leu Ser Asn Leu Lys Thr Gly Asn Asn Ile Leu Gly Leu Pro Glu
1 5 10 15

Phe Glu Leu Asn Gly Cys Arg Phe Leu Tyr Lys Lys Gly Ile Glu Lys
20 25 30

Thr Ile Ile Thr Phe Ser Ala Phe Pro Pro Lys Asp Ile Ala Gln Lys
35 40 45

Tyr Asn Tyr Ile Lys Asp Phe Leu Ser Ser Asn Tyr Thr Phe Leu Ala
50 55 60

Phe Leu Asp Thr Lys Tyr Pro Glu Asp Asp Ala Arg Gly Thr Tyr Tyr
65 70 75 80

Ile Thr Asn Glu Leu Asp Asn Gly Tyr Leu Gln Thr Ile His Cys Ile
85 90 95

Ile Gln Leu Leu Ser Asn Thr Asn Gln Glu Asp Thr Tyr Leu Leu Gly
100 105 110

Ser Ser Lys Gly Gly Val Gly Ala Leu Leu Leu Gly Leu Thr Tyr Asn
115 120 125

Tyr Pro Asn Ile Ile Ile Asn Ala Pro Gln Ala Lys Leu Ala Asp Tyr
130 135 140

Ile Lys Thr Arg Ser Lys Thr Ile Leu Ser Tyr Met Leu Gly Thr Ser
145 150 155 160

Lys Arg Phe Gln Asp Ile Asn Tyr Asp Tyr Ile Asn Asp Phe Leu Leu
165 170 175

Ser Lys Ile Lys Thr Cys Asp Ser Ser Leu Lys Trp Asn Ile His Ile
180 185 190

Thr Cys Gly Lys Asp Asp Ser Tyr His Leu Asn Glu Leu Glu Ile Leu
195 200 205

Lys Asn Glu Phe Asn Ile Lys Ala Ile Thr Ile Lys Thr Lys Leu Ile
210 215 220

Ser Gly Gly His Asp Asn Glu Ala Ile Ala His Tyr Arg Glu Tyr Phe
225 230 235 240

Lys Thr Ile Ile Gln Asn Ile *
245

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 288 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Arg Lys Ile Thr Phe Ile Ile Pro Ile Lys Gln Ser Leu Ile Lys
1 5 10 15

Pro Asp Cys Phe Ile Arg Leu Phe Phe Asn Leu Phe Leu Leu Lys Lys
20 25 30

Phe Ser Ser Lys Tyr Gly Phe Ser Ile Leu Val Ala Asp Asn Ser Asn
35 40 45

Phe Leu Trp Lys Asn Ile Ile Lys Leu Ile Thr Lys Phe Tyr Lys Cys
50 55 60

Asn Tyr Ile Ser Ile Lys Ser His Asn Thr Phe Tyr Thr Pro Ala Lys
65 70 75 80

Ile Lys Asn Ala Ala Ala Ile Tyr Ser Phe Asn Thr Leu Asn Ser Asn
85 90 95

Tyr Ile Leu Phe Leu Asp Val Asp Val Leu Leu Ser Glu Asn Phe Ile
100 105 110

Gln His Leu Ile Lys Lys Thr Lys Thr Asn Ile Ala Phe Asp Trp Tyr
115 120 125

Pro Val Ser Phe Leu Asn Lys Gln Phe Gly Ile Ile Asn Phe Ile Leu
130 135 140

Phe Ser Tyr Lys Gly Asn Leu Asn Ile Glu Glu Ser Phe Ile Ile Gln
145 150 155 160

Thr Gly Phe Val Thr Gly Leu Gln Leu Phe Asn Ser Asp Phe Phe Tyr
165 170 175

Lys Thr Ala Gly Tyr Asn Glu Ser Phe Leu Gly Tyr Gly Cys Glu Asp
180 185 190

Ile Glu Met Ile His Arg Ala Thr Leu Leu Leu Asn Ile Arg Pro Ala
195 200 205

Phe Asn Glu Asn His Gln Tyr Phe Thr Asp Asp Arg Gly Tyr Met Pro
210 215 220

Ser Lys Leu Thr Gly Phe Arg Asn Tyr Phe Tyr Tyr Leu Lys Arg Asp
225 230 235 240

Glu Phe Ser Asn Leu Gln Ile Thr Pro Lys His Phe Trp His Lys Arg
245 250 255

Lys Asn Lys Ser Lys Tyr Leu Lys Asn Arg Tyr Gln Asn Asp Val Lys
260 265 270

Met Ile Gln Ile Met Lys Asp Phe Asp Arg Lys Phe Leu Lys Asn *
275 280 285

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTGTGGAAGT TTAATTGTAG GATG 24

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCACCACCAA ACAATACTGC CG 22

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GCAATACCAT TACGTTTATC TCTC 24

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GTTTCAGGAT TGTTGATTAC TTCAGC 26

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GTCCTACGCC CTGCAGAGCT GG 22

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CATTAGGCCT AAATGCCTGA GG 22

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCTGAAGTTG TTAAACATCA AACAC 25

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCTACGACAG ATGCAAAGGC G 21

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AGAGGATTGG CTATTACATA TAGC 24

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AGCTCTGTTG TCGATTACTC TCC 23

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CATTACACAG GTTGGCTGGA AGACGG 26

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GCAGCTCGAC TTCAAATATC AAAGTGGC 28

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GCCAGCAGGA AGAAAACCTC G 21

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GCCGTTGTAG CTGTACCACG C 21

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CACCACCAAA CAATACTGCC 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GCTTGTTCAT TTGCTACCAA GTGG 24

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CCAGCATCAA TATCCTGCCA CG 22

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CCATCATTTG TGCAAGGCTG CG 22

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CATCCTACAA TTAAACTTCC ACAC 24

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAATACTAAT TATACTCTAC GTACTC 26

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 275 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTCCGGAGAT AACCTATGGG TTAAACGCCC AGGCAATGGA GACTTCAGCG TCAACGAATA 60

TGAAACATTA TTTGGTAAGG TCGCTGCTTG CAATATTCGC AAAGGTGCTC AAATCAAAAA 120

AACTGATATT GAATAAAAAT CTATAAATTG ACTCAATTTA ATGATAATCG GCTGACTTTT 180

CAGTCGATTA TCATTAAAAA TATACGGAAA AACAAATGTT GCAGAAAATA AGAAAAGCTC 240

TCTTCCACCC AAAAAAATTC TTCCAAGATT CCCAG 275

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 279 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Consensus sequence generated from comparison of SEQ ID NOs:6, 7 and 29."

(iii) HYPOTHETICAL: YES

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 191..195

(D) OTHER INFORMATION: /note= "At positions 191-195, N can be A, T, C or G or no nucleotide."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GTCCGGAGAT AACCTATGGG TTAAACGCCC AGGCAATGGA GACTTCAGCG TCAACGAATA 60

TGAAACATTA TTTGGTAAGG TCGCTGCTTG CAATATTCGC AAAGGTGCTC AAATCAAAAA 120

AACTGATATT GAATAANNNT NTATNAANTA NTNANTTTAN TNANANNNGN NTGNCTNTTN 180

NNNNNAGNNN ATTNTCATTA ANNNTNNANN GANANANNAA TGNTNNAGAA AATAANAAAA 240

GCTCTNTTNC ANCCNAAAAA NTTNTTNCAA GATTCNNNG 279

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 410 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TGAGACAACT TTTTTTGCAC TTGGGCCAGA GGAGGGAATA GCACTACATA GCACTACATG 60

CACTTCCCAA AATTAAAAAA GAAATTACAA TACAAAACTT TAACTTAAGC ATAAAATAAA 120

AAATCTCATT AAGTATGATT GTTTTTAAAT AAATTTAAAA CCTACCAGAG ATACAATACC 180

ACTTTATTTT GTAGAACACA AACGTGTATA ATATATGACA TAAACATCAT CTTCGAAATA 240

ATATTGGGGC TTAGGAAGCA AAATCATCAA AAAACGTGAT AAGCTCCTAA TATTTTTAAC 300

ACATTACTAT ATTACACATA GGATATTCCA ATGAAAGTCT TAACCGTCTT TGGCACTCGC 360

CCTGAAGCTA TTAAAATGGC GCCTGTAATT CTAGAGTTAC AAAAACATAA 410

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CCACCACCAA ACAATACTGC CG 22

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GTCAACTCAG AAGATAAGAA TTGG 24

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TCTCTTTTGT GATTCCGCTC C 21

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GAATAGCACT ACATGCACTT CCC 23

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CAGGGCGAGT GCCAAAGACG 20

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GAAGCTGTAG CTGCAGGAAC TG 22

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

AATCATTTCA ATATCTTCAC AGCC 24

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

TTACCTGAAT TTGAGTTGAA TGGC 24

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GTACCAATCA AAGGCGATAT TGG 23

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CAAAGGAAGT TACTGTTGTC TGC 23

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

TTCATATAAC TTGCGGAAAA GATG 24

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GAGCCTATTC GAAATCAAAG CTG 23

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

AGATACCATT AGTGCATCTA TGAC 24

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CATGAAACTC AGCACAGATA GAAC 24

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GTTATTTAAA TCTAGCCATG TGG 23

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CGTGGCAGGA TATTGATGCT GG 22

Claims:

1. A DNA construct comprising at least one neisserial capsular polysaccharide gene comprising, or hybridizing under stringent conditions to, a nucleotide sequence selected from the group consisting of SEQ ID NO:8 (nucleotides 479-1597; ORF1); SEQ ID NO:8 (nucleotides 1599-3236; ORF2); SEQ ID NO:8 (nucleotides 3309-4052; ORF3); and SEQ ID NO:8 (nucleotides 4054-4917; ORF4).

2. A purified nucleic acid preparation comprising at least about 15 bases of a nucleotide sequence of a serogroup A *Neisseria meningitidis* strain F8229 gene selected from the group consisting of SEQ ID NO:8 (nucleotides 479-1597), SEQ ID NO:8 (nucleotides 1599-3236), SEQ ID NO:8 (nucleotides 3309-4052) and SEQ ID NO:8 (nucleotides 4054-4917).

3. A kit useful in the detection of a strain of *Neisseria meningitidis* of serogroup A, comprising the purified nucleic acid preparation of claim 2.

4. A method of serogroup typing or screening for the presence of a serogroup A marker in a sample comprising a strain of *Neisseria meningitidis*, comprising the step of contacting said sample with the nucleic acid preparation of claim 2 under stringent hybridization conditions such that the presence of a gene specifying said serogroup A marker is revealed in said sample by hybridizing to said nucleic acid preparation.

5. A recombinant DNA preparation derived from a strain of *Neisseria meningitidis* of a first serogroup comprising a capsule switching mutation such that a capsular polysaccharide of a second serogroup is expressed, wherein one of said serogroups is serogroup A.

6. The recombinant DNA preparation of claim 5 wherein said neisserial strain is a naturally occurring isolate or a genetically engineered strain.

7. The recombinant DNA preparation of claim 5 wherein said first serogroup and said second serogroup type are selected from the group consisting of serogroups A, B, C, Y and W-135, with the provisos that the first serogroup is different from the second serogroup.

8. The recombinant DNA preparation strain of claim 5 wherein said neisserial strain is selected from the group of *Neisseria meningitidis* serogroups consisting of serogroup B: ET-301-1070, Et-301-1069, NMB-43, NMB-M7; serogroup C: FAM18-43, FAM18-47, 1205, 1205-43, 1205-43CC, 1205-M7, 1198, 1204; serogroup Y: GA0929, GA0929-43, GA0929-M7; serogroup W-135: GA1002, GA1002-43, GA1002-M7; serogroup A: F8229, F8239, F8239-43 and F8239-M7.

9. The recombinant DNA preparation of claim 5 wherein said strain comprises a plurality of capsule switching mutations such that a plurality of capsular polysaccharides, each being a marker of a different serogroup type, is expressed.

10. The recombinant DNA preparation of claim 8 wherein a capsular polysaccharide biosynthetic coding region comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:8 (nucleotides 479-1597), SEQ ID NO:8 (nucleotides 1599-3236), SEQ ID NO:8 (nucleotides 3309-4052) and SEQ ID NO:8 (nucleotides 4054-4917).

11. The DNA preparation of claim 10 wherein said DNA comprises at SEQ ID NO:8 (nucleotides 479-1597), SEQ ID NO:8 (nucleotides 1599-3236), SEQ ID NO:8 (nucleotides 3309-4052) and SEQ ID NO:8 (nucleotides 4054-4917) as a biosynthesis genetic cassette determining serogroup specificity.

12. The DNA preparation of claim 10 wherein said genetic cassette is recombined such that the resultant recombination expresses a serogroup specificity different from that expressed prior to said recombination.

13. An immunogenic composition comprising a capsular polysaccharide derived from the expression of the recombinant DNA preparation of any of claims 5-12, an adjuvant and a suitable carrier.

14. A method of preparing a vaccine for meningococcal disease comprising the steps of:

(a) expressing the capsular polysaccharide encoded in the recombinant DNA preparation of any of claims 5-12; and

(b) combining said capsular polysaccharide with an adjuvant and suitable carrier

such that said vaccine is prepared from a substantially pure capsular polysaccharide.

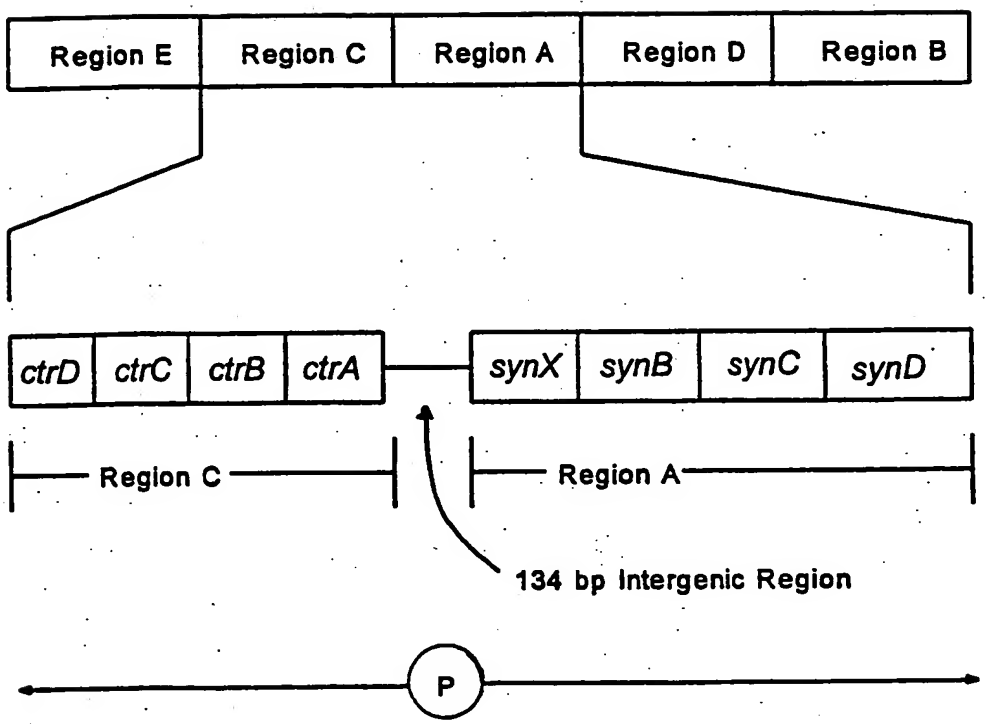

Region E
Region C
Region A
Region D
Region B
ctrD
ctrC
ctrB
ctrA
synX
synB
synC
synD
Region C
Region A
134 bp Intergenic Region
P

FIG. 1A

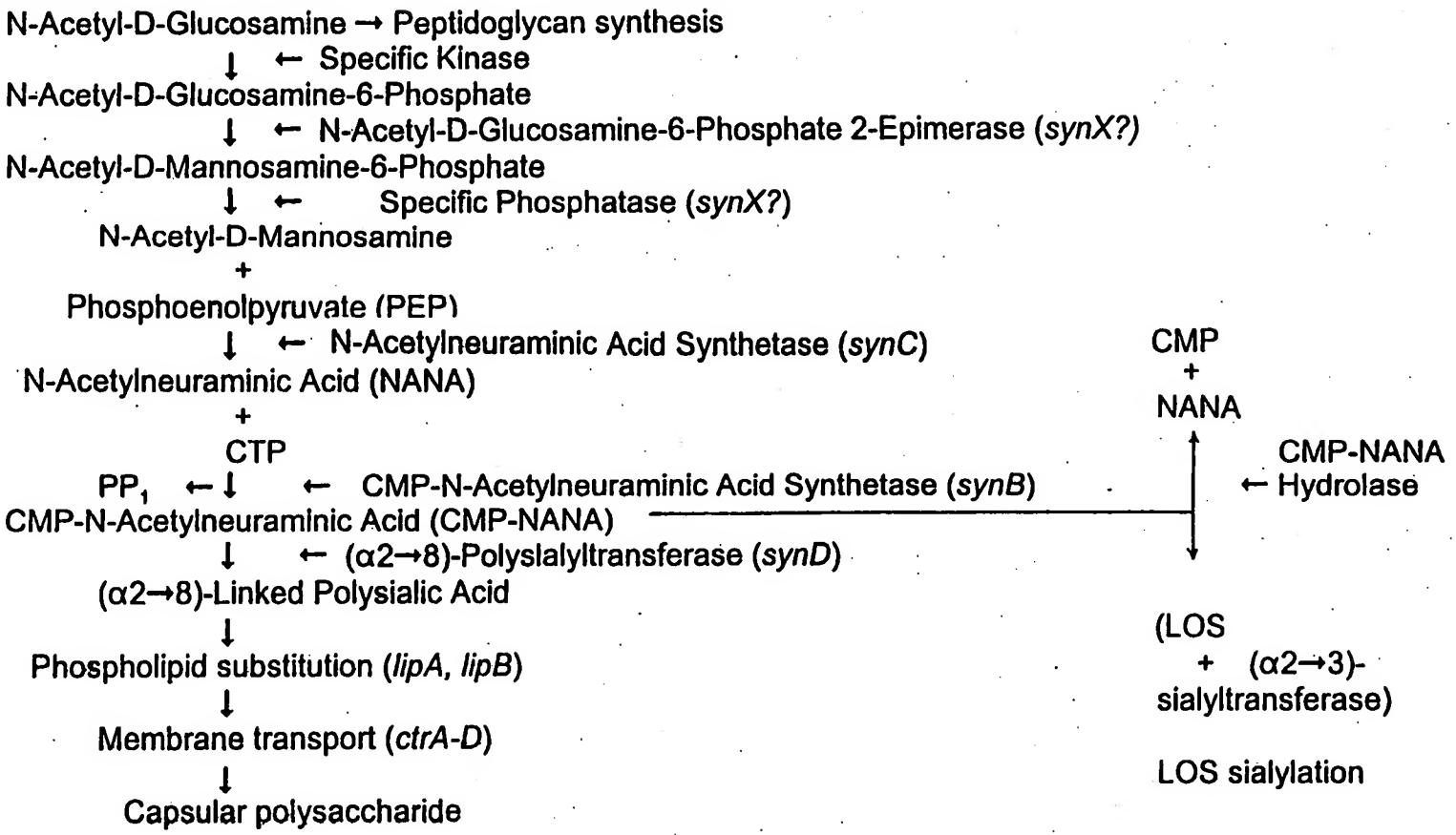

N-Acetyl-D-Glucosamine → Peptidoglycan synthesis
↓ ← Specific Kinase
N-Acetyl-D-Glucosamine-6-Phosphate
↓ ← N-Acetyl-D-Glucosamine-6-Phosphate 2-Epimerase (synX?)
N-Acetyl-D-Mannosamine-6-Phosphate
↓ ← Specific Phosphatase (synX?)
N-Acetyl-D-Mannosamine
+
Phosphoenolpyruvate (PEP)
↓ ← N-Acetylneuraminic Acid Synthetase (synC)
N-Acetylneuraminic Acid (NANA)
+
CTP
PP1 ← ↓ ← CMP-N-Acetylneuraminic Acid Synthetase (synB)
CMP-N-Acetylneuraminic Acid (CMP-NANA)
↓ ← (α2→8)-Polysialyltransferase (synD)
(α2→8)-Linked Polysialic Acid
↓
Phospholipid substitution (lipA, lipB)
↓
Membrane transport (ctrA-D)
↓
Capsular polysaccharide
CMP
+
NANA
CMP-NANA
← Hydrolase
(LOS
+ (α2→3)-
sialyltransferase)
LOS sialylation

FIG. 1B

FIG. 1C

- BMB Ladder II
- NMB
- GC
- C
- W-135
- Y
- C-301*
- C-301
- B-301*
- A
- A
- *N. Lactamica*
- BMB Ladder VI

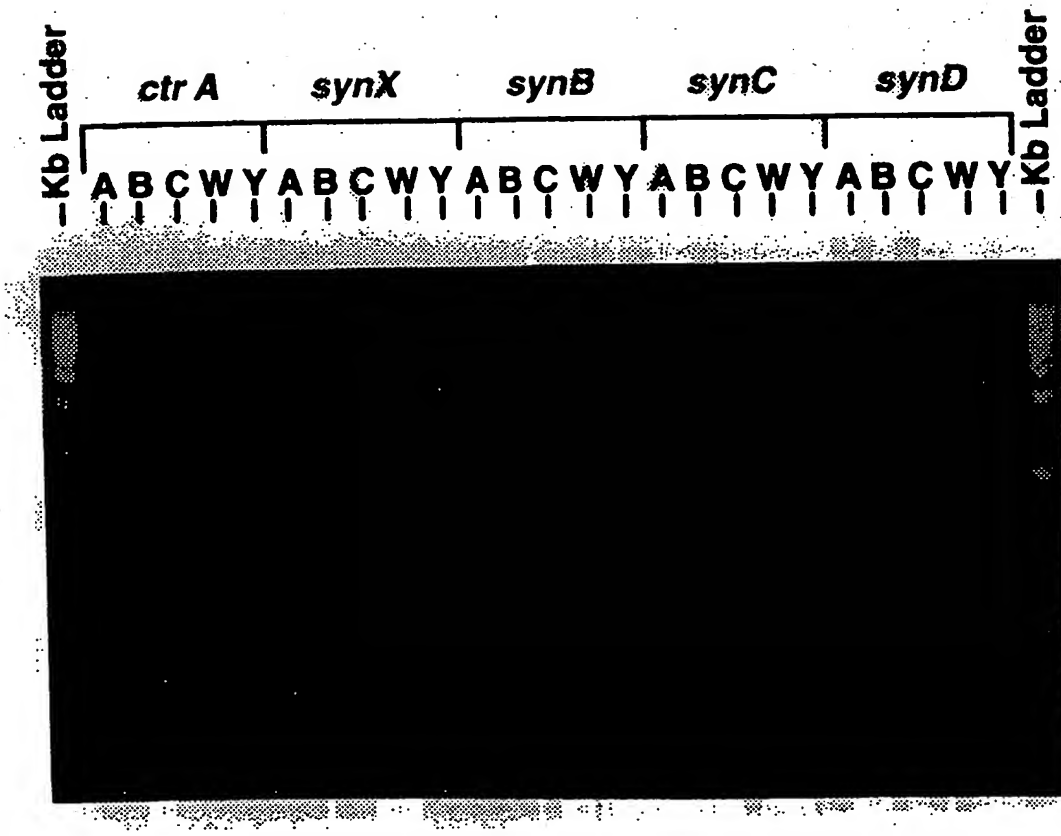

Kb Ladder
ctr A
synX
synB
synC
synD
A B C W Y A B C W Y A B C W Y A B C W Y A B C W Y
Kb Ladder

FIG. 1D

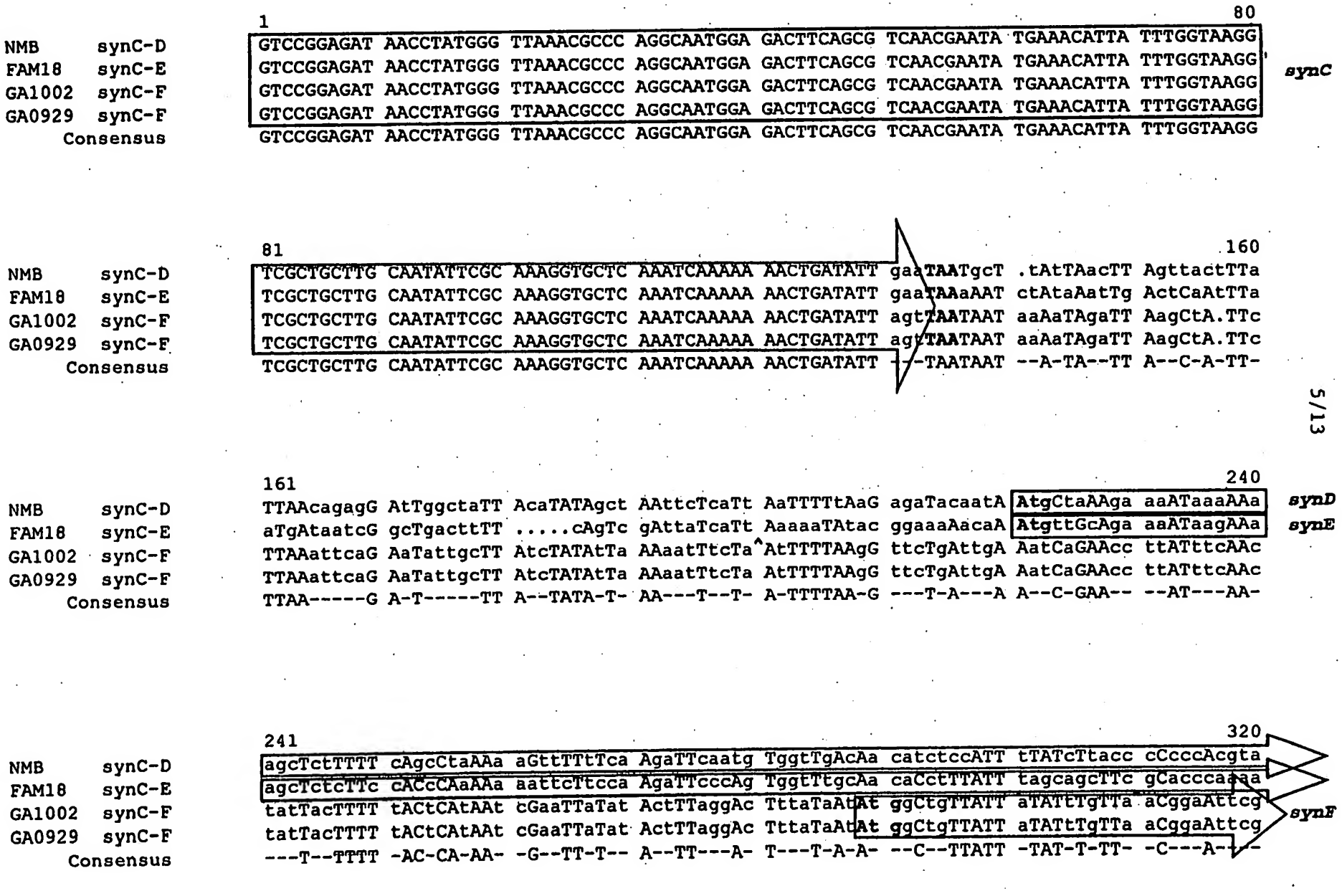

1 80
NMB synC-D GTCCGGAGAT AACCTATGGG TTAAACGCCC AGGCAATGGA GACTTCAGCG TCAACGAATA TGAAACATTA TTTGGTAAGG
FAM18 synC-E GTCCGGAGAT AACCTATGGG TTAAACGCCC AGGCAATGGA GACTTCAGCG TCAACGAATA TGAAACATTA TTTGGTAAGG
GA1002 synC-F GTCCGGAGAT AACCTATGGG TTAAACGCCC AGGCAATGGA GACTTCAGCG TCAACGAATA TGAAACATTA TTTGGTAAGG
GA0929 synC-F GTCCGGAGAT AACCTATGGG TTAAACGCCC AGGCAATGGA GACTTCAGCG TCAACGAATA TGAAACATTA TTTGGTAAGG
Consensus GTCCGGAGAT AACCTATGGG TTAAACGCCC AGGCAATGGA GACTTCAGCG TCAACGAATA TGAAACATTA TTTGGTAAGG
synC
81 160
NMB synC-D TCGCTGCTTG CAATATTCGC AAAGGTGCTC AAATCAAAAA AACTGATATT gaaTAATgcT .tAtTAacTT AgttactTTa
FAM18 synC-E TCGCTGCTTG CAATATTCGC AAAGGTGCTC AAATCAAAAA AACTGATATT gaaTAAaAAT ctAtaAatTg ActCaAtTTa
GA1002 synC-F TCGCTGCTTG CAATATTCGC AAAGGTGCTC AAATCAAAAA AACTGATATT agtTAATAAT aaAaTAgaTT AagCtA.TTc
GA0929 synC-F TCGCTGCTTG CAATATTCGC AAAGGTGCTC AAATCAAAAA AACTGATATT agtTAATAAT aaAaTAgaTT AagCtA.TTc
Consensus TCGCTGCTTG CAATATTCGC AAAGGTGCTC AAATCAAAAA AACTGATATT ---TAATAAT --A-TA--TT A--C-A-TT-
161 240
NMB synC-D TTAAcagagG AtTggctaTT AcaTATAgct AAttcTcaTt AaTTTTtAaG agaTacaatA AtgCtaAAga aaATaaaAAa
FAM18 synC-E aTgAtaatcG gcTgacttTT .....cAgTc gAttaTcaTt AaaaaTAtac ggaaaAacaA AtgttGcAga aaATaagAAa
GA1002 synC-F TTAAattcaG AaTattgcTT AtcTATAtTa AAaatTtcTa^AtTTTTAAgG ttcTgAttgA AatCaGAAcc ttATttcAAc
GA0929 synC-F TTAAattcaG AaTattgcTT AtcTATAtTa AAaatTtcTa AtTTTTAAgG ttcTgAttgA AatCaGAAcc ttATttcAAc
Consensus TTAA-----G A-T-----TT A--TATA-T- AA---T--T- A-TTTTAA-G ---T-A---A A--C-GAA-- --AT---AA-
synD
synE
241 320
NMB synC-D agcTctTTTT cAgcCtaAAa aGttTTtTca AgaTTcaatg TggtTgAcAa catctccATT tTATcTtacc cCcccAcgta
FAM18 synC-E agcTctcTTc cACcCAaAAa aattcTtcca AgaTTcccAg TggtTTgcAa caCctTTATT tagcagcTTc gCacccaaaa
GA1002 synC-F tatTacTTTT tACtCAtAAt cGaaTTaTat ActTTaggAc TttaTaAtAt ggCtgTTATT aTATtTgTTa aCggaAttcg
GA0929 synC-F tatTacTTTT tACtCAtAAt cGaaTTaTat ActTTaggAc TttaTaAtAt ggCtgTTATT aTATtTgTTa aCggaAttcg
Consensus ---T--TTTT -AC-CA-AA- -G--TT-T-- A--TT---A- T---T-A-A- --C--TTATT -TAT-T-TT- -C---A---
synF

FIG. 2

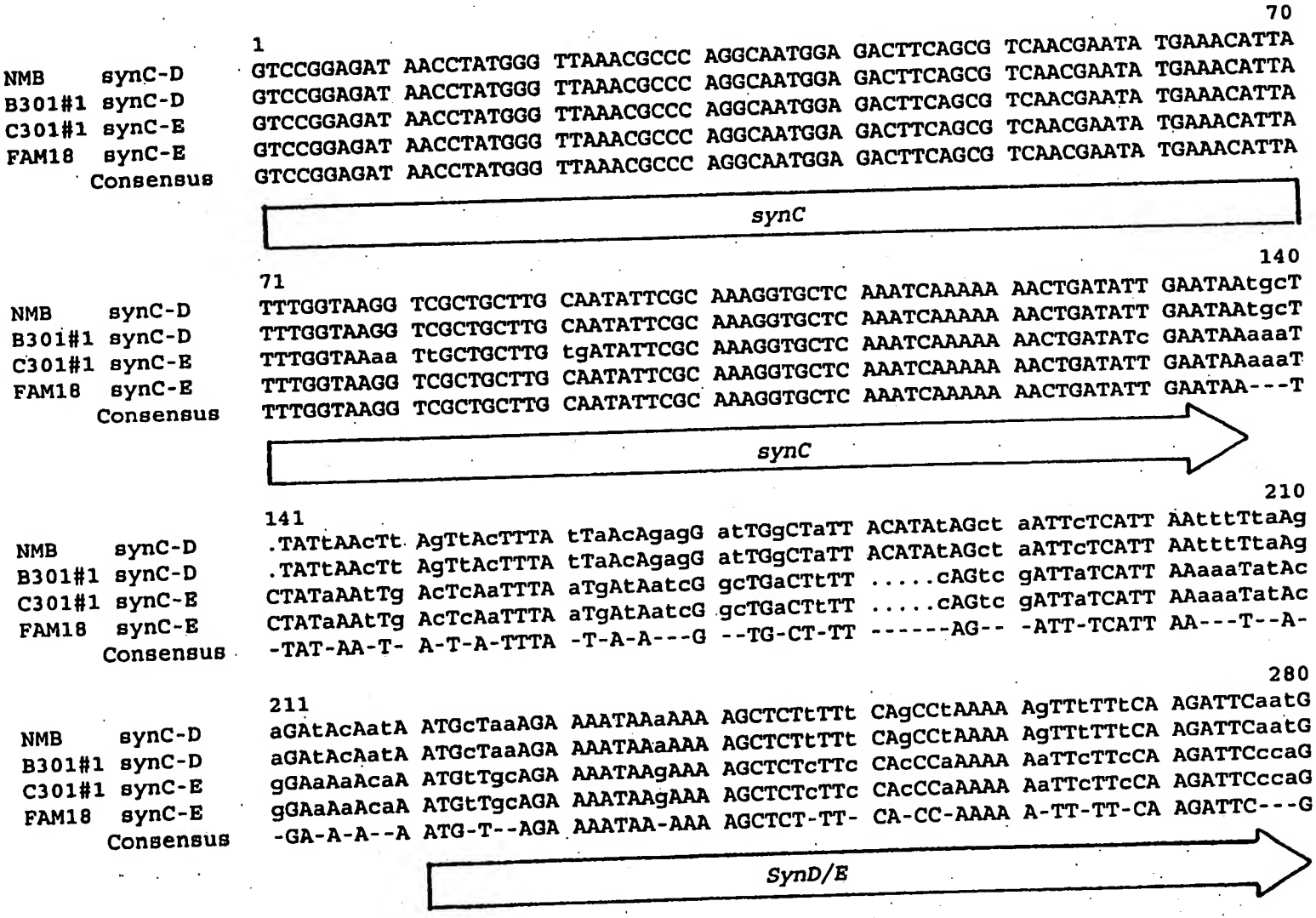

70
1
NMB synC-D GTCCGGAGAT AACCTATGGG TTAAACGCCC AGGCAATGGA GACTTCAGCG TCAACGAATA TGAAACATTA
B301#1 synC-D GTCCGGAGAT AACCTATGGG TTAAACGCCC AGGCAATGGA GACTTCAGCG TCAACGAATA TGAAACATTA
C301#1 synC-E GTCCGGAGAT AACCTATGGG TTAAACGCCC AGGCAATGGA GACTTCAGCG TCAACGAATA TGAAACATTA
FAM18 synC-E GTCCGGAGAT AACCTATGGG TTAAACGCCC AGGCAATGGA GACTTCAGCG TCAACGAATA TGAAACATTA
Consensus GTCCGGAGAT AACCTATGGG TTAAACGCCC AGGCAATGGA GACTTCAGCG TCAACGAATA TGAAACATTA
synC
140
71
NMB synC-D TTTGGTAAGG TCGCTGCTTG CAATATTCGC AAAGGTGCTC AAATCAAAAA AACTGATATT GAATAAtgcT
B301#1 synC-D TTTGGTAAGG TCGCTGCTTG CAATATTCGC AAAGGTGCTC AAATCAAAAA AACTGATATT GAATAAtgcT
C301#1 synC-E TTTGGTAAaa TtGCTGCTTG tgATATTCGC AAAGGTGCTC AAATCAAAAA AACTGATATc GAATAAaaaT
FAM18 synC-E TTTGGTAAGG TCGCTGCTTG CAATATTCGC AAAGGTGCTC AAATCAAAAA AACTGATATT GAATAAaaaT
Consensus TTTGGTAAGG TCGCTGCTTG CAATATTCGC AAAGGTGCTC AAATCAAAAA AACTGATATT GAATAA---T
synC
210
141
NMB synC-D .TATtAAcTt AgTtAcTTTA tTaAcAgagG atTGgCTaTT ACATAtAGct aATTcTCATT AAtttTtaAg
B301#1 synC-D .TATtAAcTt AgTtAcTTTA tTaAcAgagG atTGgCTaTT ACATAtAGct aATTcTCATT AAtttTtaAg
C301#1 synC-E CTATaAAtTg AcTcAaTTTA aTgAtAatcG gcTGaCTtTT .....cAGtc gATTaTCATT AAaaaTatAc
FAM18 synC-E CTATaAAtTg AcTcAaTTTA aTgAtAatcG gcTGaCTtTT .....cAGtc gATTaTCATT AAaaaTatAc
Consensus -TAT-AA-T- A-T-A-TTTA -T-A-A---G --TG-CT-TT ------AG-- -ATT-TCATT AA---T--A-
280
211
NMB synC-D aGAtAcAatA ATGcTaaAGA AAATAAaAAA AGCTCTtTTt CAgCCtAAAA AgTTtTTtCA AGATTCaatG
B301#1 synC-D aGAtAcAatA ATGcTaaAGA AAATAAaAAA AGCTCTtTTt CAgCCtAAAA AgTTtTTtCA AGATTCaatG
C301#1 synC-E gGAaAaAcaA ATGtTgcAGA AAATAAgAAA AGCTCTcTTc CAcCCaAAAA AaTTcTTcCA AGATTCccaG
FAM18 synC-E gGAaAaAcaA ATGtTgcAGA AAATAAgAAA AGCTCTcTTc CAcCCaAAAA AaTTcTTcCA AGATTCccaG
Consensus -GA-A-A--A ATG-T--AGA AAATAA-AAA AGCTCT-TT- CA-CC-AAAA A-TT-TT-CA AGATTC---G
SynD/E

FIG. 3A

			1 34444444444 557888
			7 52233333334 397114
			5 16834567890 587346
B301 #1	(B)	*ctrA-synX*	A CAT..... CAGGCA
B301 #2	(B)	*ctrA-synX*	- ---------- ------
C301 #1	(C)	*ctrA-synX*	- ---------- TGAATG
C301 #2	(C)	*ctrA-synX*	- ---------- TGAATG
C301 #3	(C)	*ctrA-synX*	- ---------- TGAATG
NMB	(B)	*ctrA-synX*	- ---TACTTATA ---ATG
FAM18	(C)	*ctrA-synX*	- ---TACTTATA ---ATG
GA0929	(Y)	*ctrA-synX*	- ---TACTTATA ---ATG
GA1002	(W)	ctrA-synX	G ---TACTTATA ---ATG
6083	(W)	*ctrA-synX*	- -GC-------- ---ATG

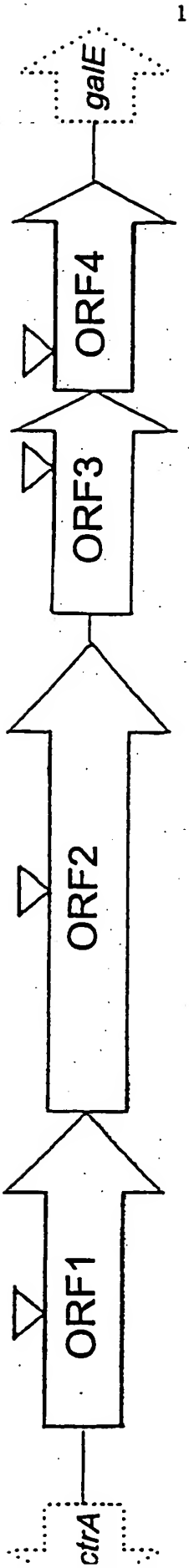
FIG. 3B-1

```
                          1111111111111111112222222222222222222
                          0223344555666777890002223334445566778
                          2092547069258127984782681470564714091

B301 #1     (B)   fkbp ACCCGCCGTCAACCACCCGAGGACCTGAGCCACGCCC
B301 #2     (B)   fkbp ----------------------------------------
C301 #1     (C)   fkbp ----------------------------------------
C301 #2     (C)   fkbp ----------------------------------------
C301 #3     (C)   fkbp ----------------------------------------
GA0929      (Y)   fkbp ----------------------G-----------------
F8239       (A)   fkbp --------C--------A-------------------
GA1002      (W)   fkbp ------------------A-------------G---T-
6083        (W)   fkbp C-------C--------A-G-------------C---
NMB         (B)   fkbp C-------C--------A-G-------------C---
M986        (B)   fkbp C-------C--------A-G-------------C---
2996        (B)   fkbp C-------C--------A-G-------------C---
C114        (B)   fkbp C-------C--------A-G-------------.---
KB          (B)   fkbp T-------C--------A--A-----T-CT--TC---
269B        (B)   fkbp T-------C--------A--A-----T-CT--TC---
FAM18       (C)   fkbp T-------C--------A--A-----T-CT--TC---

N.  ciner.        fkbp --A-A--AC---T--T-------------T--TC---
N.  lact.         fkbp T--------------T-----A----TGCT--TCT--
N.  poly.         fkbp -T-------------T-A--------T-CTT-TCT--
N.  elong.        fkbp T--TA--TC-C-GT-T--A-----T-C-----TC-T-
N.  sicca         fkbp C-TT-GT-CGTG-TC-T------T-G-----G-----
N.  flav.         fkbp T--TA--TC-C-GT-T--A-----T-C-----TC-T-
N.  subfl.        fkbp T--TA--TC-C-GT-T--A-----T-C---A-TA-TT
```

FIG. 3B-2

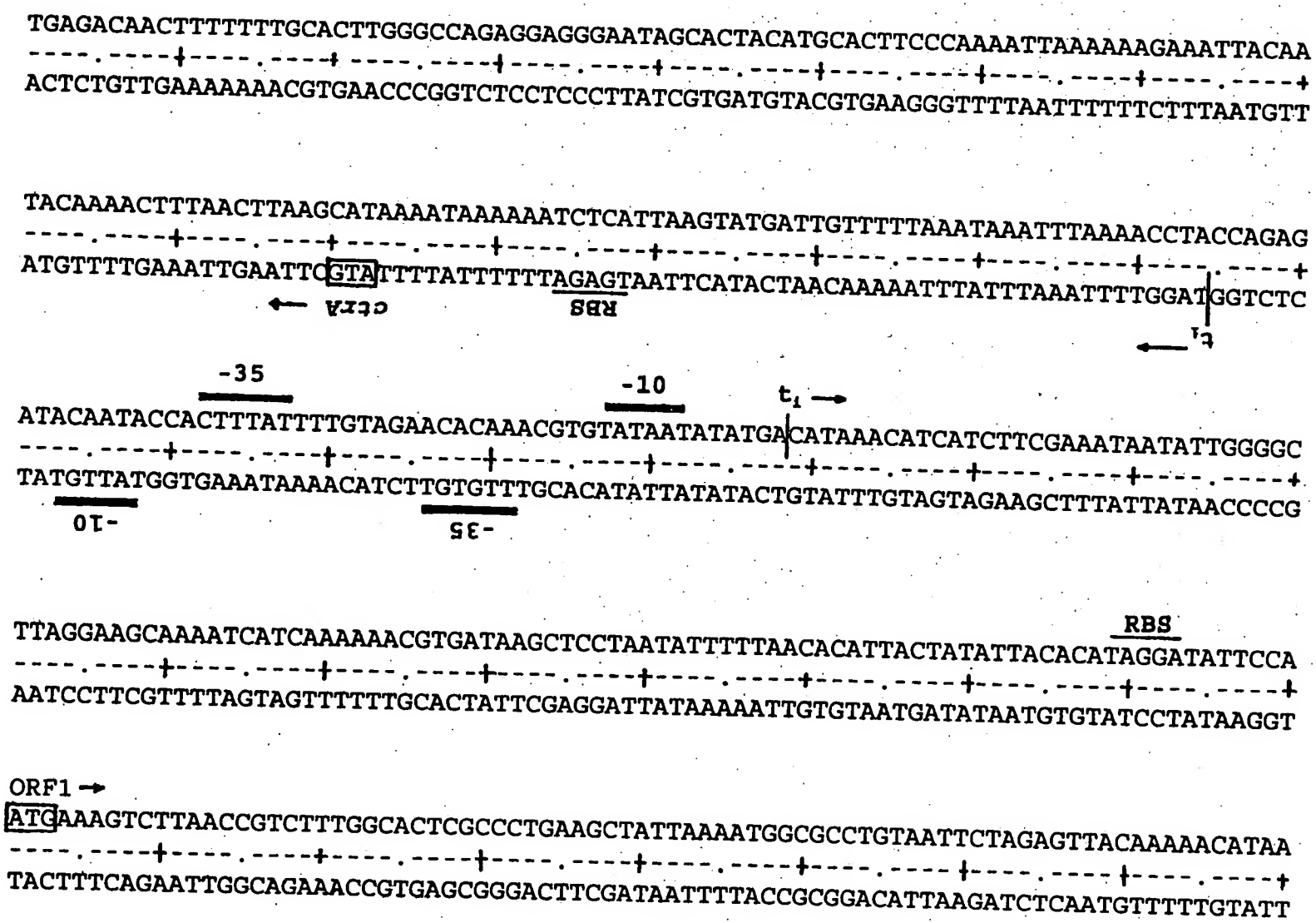
```
                          2233333333344455555556666777888899
                          8933355566728922333472469255348956 8
                          2703945713590525147491800039408149 1

B301 #1     (B)   recA CTCACCCCCCCCGCCACTGACTCCCGGGTAGGGGC
B301 #2     (B)   recA -----------------------------------
C301 #1     (C)   recA -----------------------------------
C301 #2     (C)   recA -----------------------------------
C301 #3     (C)   recA -----------------------------------
Nm-HF46     (A)   recA -----------------------------------
NMB         (B)   recA -----------------------------------
Nm-44/76    (B)   recA -----------------------------------
FAM18       (C)   recA -----------------------------------
Nm-N94II    (Y)   recA ---------------------------ACCGCC---
F8239       (A)   recA ---C-------T--------------ACCGCC---
Nm-S3446    (B)   recA ---C-------T--------------ACCGCC---
Nm-HF130    (B)   recA ---C-------T-T----A---T---ACC-CC---
Ng-FA19           recA -C-C-A---------G-----CT-TA--C-CC---
Nm-M470     (B)   recA ---C--------C-----A---TT--ACCGCC--T
GA0929      (Y)   recA ---C--------C-----A---TT--ACCGCC--T
GA1002      (W)   recA AC-C-A-----T------A---T---ACC-CC-A-
Nm-HF116    (Z)   recA AC-C-A-----T------A---T---ACC-CCA--
Nm-P63      (B)   recA A-TCTATATGA-C-TGGCCGTC-T--ACC-CC-A-
```

FIG. 3B-3



0.5 kb

FIG. 4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ACTCTGTTGAAAAAAACGTGAACCCGGTCTCCTCCCTTATCGTGATGTACGTGAAGGGTTTTAATTTTTTCTTTAATGTT
TACAAAACTTTAACTTAAGCATAAAATAAAAAATCTCATTAAGTATGATTGTTTTTAAATAAATTTAAAACCTACCAGAG
ATGTTTTGAAATTGAATTCGTATTTTATTTTTTAGAGTAATTCATACTAACAAAAATTTATTTAAATTTTGGATGGTCTC
-35
-10
ATACAATACCACTTTATTTTGTAGAACACAAACGTGTATAATATATGACATAAACATCATCTTCGAAATAATATTGGGGC
TATGTTATGGTGAAATAAAACATCTTGTGTTTGCACATATTATATACTGTATTTGTAGTAGAAGCTTTATTATAACCCCG
RBS
TTAGGAAGCAAAATCATCAAAAAACGTGATAAGCTCCTAATATTTTTAACACATTACTATATTACACATAGGATATTCCA
AATCCTTCGTTTTAGTAGTTTTTTGCACTATTCGAGGATTATAAAAATTGTGTAATGATATAATGTGTATCCTATAAGGT
ORF1
ATGAAAGTCTTAACCGTCTTTGGCACTCGCCCTGAAGCTATTAAAATGGCGCCTGTAATTCTAGAGTTACAAAAACATAA
TACTTTCAGAATTGGCAGAAACCGTGAGCGGGACTTCGATAATTTTACCGCGGACATTAAGATCTCAATGTTTTTGTATT

FIG. 5

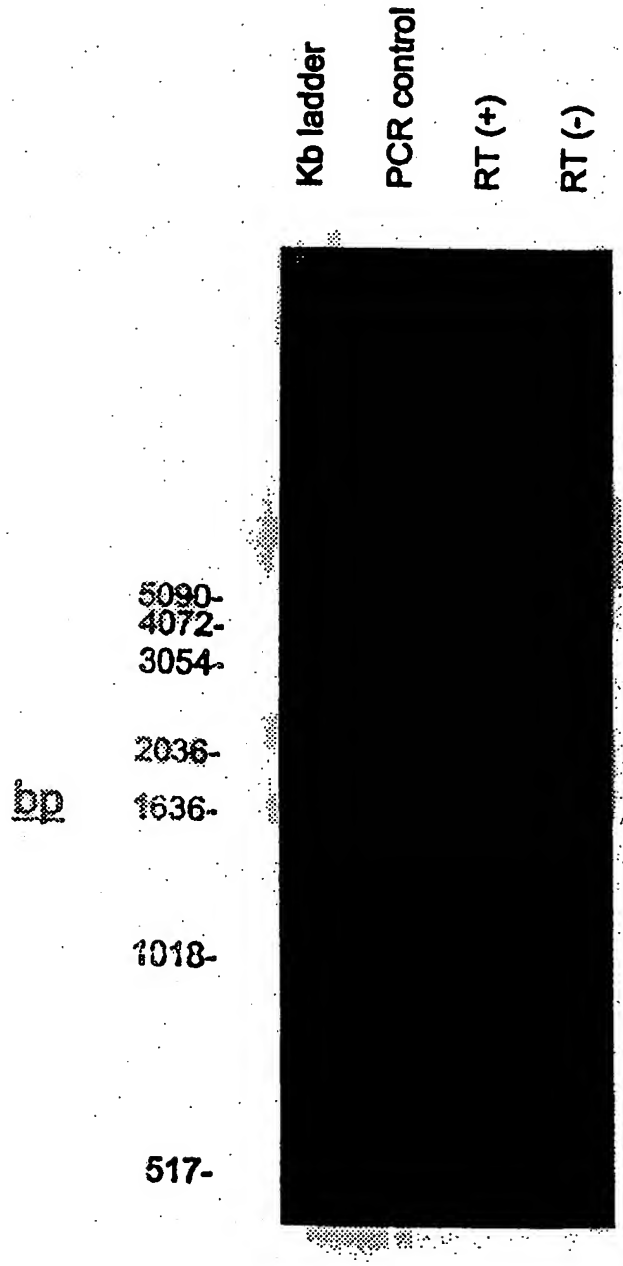
Kb ladder
PCR control
RT (+)
RT (-)
5090-
4072-
3054-
2036-
bp
1636-
1018-
517-

FIG. 6

FIG. 7B

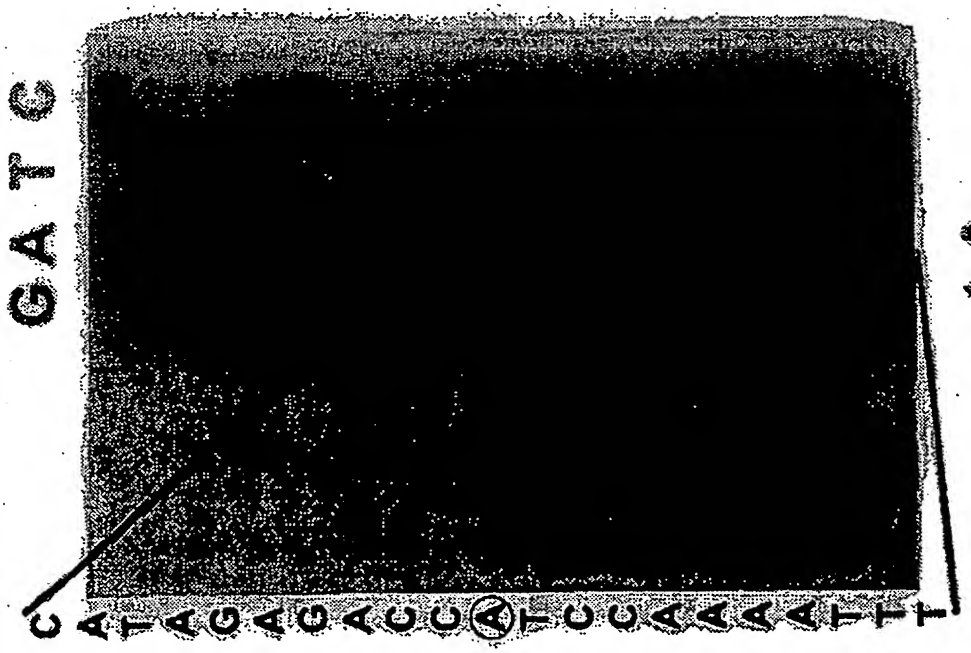

FIG. 7A

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/06946

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/02, 21/04; C12Q 1/68; C12P 21/06; A61K 39/05

US CL :536/23.1, 23.7; 435/6, 69.1; 424/250.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 23.7; 435/6, 69.1; 424/250.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, GENBANK, EMBL53

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FROSCH et al. Conserved Outer Membrane Protein of Neisseria meningitidis Involved in Capsule Expression. Infection and Immunity. March 1992. Vol.60. No.3., pages 798-803, see entire document.	1-14
A	SWARTLEY et al. Capsule Switching of Neisseria meningitidis. Proc. Natl. Acad. Sci. USA. January 1997. Vol. 94. pages 271-276, see entire document.	1-14
A	GANGULI et al. Molecular Cloning and Analysis of Genes for Sialic Acid Synthesis in Neisseria meningitidis Group B and Purification of the Meningococcal CMP-NeuNAc Synthetase Enzyme. August 1994. Vol. 176. No. 15. pages 4583-4589, see entire document.	1-14

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
18 MAY 1998	18 AUG 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks	Authorized officer

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/06946

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FROSCH et al. Evidence for a Common Molecular Origin of the Capsule Gene Loci in Gram-negative Bacteria Expressing Group II Capsular Polysaccharide. Vol. 5. No. 5. pages 1251-1263, see entire document.	1-14